A STUDY ON PESTICIDE DEGRADATION BY SOIL BACTERIA IN TEA AND COFFEE PLANTATIONS AT VALPARAI ESTATE REGION OF WESTERN GHATS

Thesis Submitted to the University of Calicut in partial fulfillment of the requirements

For the award of the Degree of

DOCTOR OF PHILOSOPHY in ZOOLOGY

By

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2024

DECLARATION

I hereby declare that the thesis entitled " ***A Study on Pesticide Degradation by Soil Bacteria in Tea and Coffee Plantations at Valparai Estate Region of Western Ghats*** " submitted to the University of Calicut for the award of Doctor Of Philosophy in Zoology is a bonafide research work done by me under the supervision and guidance of Dr. Vimala K John, Associate Professor, Research and Post Graduate Department of Zoology, St. Thomas' College (Autonomous), Thrissur.

I also declare that the findings presented in this thesis are original and do not form the basis for the award of any other degree, diploma, or other similar titles from any other university.

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CERTIFICATE

This is to certify that the thesis entitled ***"A Study on Pesticide Degradation by Soil Bacteria in Tea and Coffee Plantations at Valparai Estate Region of Western Ghats"*** submitted to the University of Calicut for the award of Doctor of Philosophy is a bonafide account of research work carried out by **Ms. Josna Victoria K Johnson** under my supervision. The work has not been submitted either partially or fully to any other university or Institution for the award of any degree, diploma, fellowship, title, or recognition.

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ABSTRACT

In the current thesis, an effort is made to investigate the potential for local tea garden bacterial isolates to biodegrade a number of pesticides that are routinely used in agricultural fields. The composite sampling technique was used to gather soil samples from eight separate sampling locations. The soil samples' physiochemical characteristics were examined. the relationship between bacterial population and pesticide residues in sampling sites was also investigated. The bacterial population and pesticide fate in the environment are affected by physiochemical factors, particularly soil pH. Ten native bacterial isolates (DRNB1, EON2, SFN1, TXM1, F1T, Q1T, GLYB2, PTEB2, FXE1, and SFT1) were isolated and evaluated for degradation studies from pesticide-applied tea plantation areas. All the bacterial isolates are characterized by molecular analysis and the phylogenetic tree was also constructed using MEGA X. Through a disc diffusion assay, isolates were evaluated for their ability to tolerate up to 500 ppm concentrations of eight different pesticides (Deltamethrin, Spiromesifen, ethion, Thiamethoxam, Quinalphos, Fenpyroximate, Glyphosate, and Propargite). For tests on pesticide degradation, isolates that could withstand pesticide concentrations up to 500 ppm were used. The formation of biosurfactants, enzyme activity, and biofilm was also investigated in the bacterial isolates. The growth of bacterial isolates in carbon, nitrogen, and pesticide sources was examined. The optimization of parameters for pesticide degradation studies was done using the Taguchi optimization methodology. The metabolites formed through the pesticide degradation were analyzed by GC-MS analysis. A microbial consortium (PDMC1-Pesticide Degrading Microbial Consortium) was created for the pesticide deltamethrin's degradation, and about 91% of the pesticide's degradation in MSM was observed. DRNB1-*Stenotrophomonas maltophilia,* SFN1-*Stenotrophomonas [Pseudomonas] geniculate*, and FXE1-*Pseudomonas aeruginosa* are the three bacterial isolates that make up the consortium.

Keywords : Pesticides, Degradation, Bio-degradation, Microbes, Microbial consortium

നിലവിലെ പ്രബന്ധത്തിൽ, കാർഷിക മേഖലകളിൽ പതിവായി ഉപയോഗിക്കുന്ന നിരവധി കീടനാശിനികൾ ജൈവവിഘടനം ചെയ്യാനുള്ള പ്രാദേശിക തേയിലത്തോട്ടത്തിലെ ബാക്ടീരിയൽ ഒറ്റപ്പെടലുകൾക്കുള്ള സാധ്യതയെക്കുറിച്ച് അന്വേഷിക്കാനുള്ള ശ്രമം നടത്തുന്നു. എട്ട് വ്യത്യസ്ത സ്ഥലങ്ങളിൽ നിന്ന് മണ്ണ് സാമ്പിളുകൾ ശേഖരിക്കാൻ കോമ്പോസിറ്റ് സാമ്പിൾ ടെക്നിക് ഉപയോഗിച്ചു. മണ്ണിന്റെ സാമ്പിളുകളുടെ ഭൗതിക രാസ സവിശേഷതകൾ പരിശോധിച്ചു. സാമ്പിൾ സൈറ്റുകളിലെ ബാക്ടീരിയകളുടെ ജനസംഖ്യയും കീടനാശിനി അവശിഷ്ടങ്ങളും തമ്മിലുള്ള ബന്ധവും അന്വേഷിച്ചു. പരിസ്ഥിതിയിലെ ബാക്ടീരിയകളുടെ ജനസംഖ്യയും കീടനാശിനിയുടെ വിധിയും ഫിസിയോകെമിക്കൽ ഘടകങ്ങളാൽ, പ്രത്യേകിച്ച് മണ്ണിന്റെ pH-നെ ബാധിക്കുന്നു. കീടനാശിനി പ്രയോഗിച്ച തേയിലത്തോട്ട പ്രദേശങ്ങളിൽ നിന്നുള്ള ഡീഗ്രേഡേഷൻ പഠനങ്ങൾക്കായി പത്ത് നേറ്റീവ് ബാക്ടീരിയൽ സ്ട്രെയിനുകൾ (DRNB1, EON2, SFN1, TXM1, F1T, Q1T, GLYB2, PTEB2, FXE1, SFT1) വേർതിരിച്ച് വിലയിരുത്തി. എല്ലാ ബാക്റ്റീരിയൽ ഐസൊലേറ്റുകളും തന്മാത്രാ വിശകലനം മുഖേനയുള്ളതാണ്, കൂടാതെ ഫൈലോജെനെറ്റിക് ട്രീയും MEGA X ഉപയോഗിച്ചാണ് നിർമ്മിച്ചിരിക്കുന്നത്. എട്ട് വ്യത്യസ്ത കീടനാശിനികളുടെ (ഡെൽറ്റാമെത്രിൻ, സ്പിറോമെസിഫെൻ, എത്തയോൺ, തിയാമെത്തോക്സം, ക്വിനൽഫോസ്, ഫെൻപൈറോക്സിമേറ്റ്, ഗ്ലൈഫോസേറ്റ്, പ്രോപ്പർഗൈറ്റ്) 500 പിപിഎം സാന്ദ്രത വരെ സഹിക്കുന്നതിനുള്ള കഴിവ് ഒരു ഡിസ്ക് ഡിഫ്യൂഷൻ അസെയിലൂടെ ഐസൊലേറ്റുകളെ വിലയിരുത്തി. കീടനാശിനി ഡീഗ്രഡേഷനെക്കുറിച്ചുള്ള പരിശോധനകൾക്കായി, 500 പിപിഎം വരെ കീടനാശിനി സാന്ദ്രതയെ ചെറുക്കാൻ കഴിയുന്ന ഐസൊലേറ്റുകൾ ഉപയോഗിച്ചു. ബയോസർഫക്റ്റന്റുകളുടെ രൂപീകരണം, എൻസൈം പ്രവർത്തനം, ബയോഫിലിം എന്നിവയും ബാക്ടീരിയൽ ഐസൊലേറ്റുകളിൽ അന്വേഷിച്ചു. കാർബൺ, നൈട്രജൻ, കീടനാശിനി സ്രോതസ്സുകൾ എന്നിവയിലെ ബാക്ടീരിയൽ ഐസൊലേറ്റുകളുടെ വളർച്ച പരിശോധിച്ചു. കീടനാശിനി ഡീഗ്രേഡേഷൻ പഠനങ്ങൾക്കായുള്ള പാരാമീറ്ററുകളുടെ ഒപ്റ്റിമൈസേഷൻ ടാഗുച്ചി ഒപ്റ്റിമൈസേഷൻ രീതി ഉപയോഗിച്ചാണ് നടത്തിയത്. കീടനാശിനി ഡീഗ്രേഡേഷൻ വഴി രൂപപ്പെട്ട മെറ്റബോളിറ്റുകളെ ജിസി-എംഎസ് വിശകലനം വിശകലനം ചെയ്തു. ഡെൽറ്റാമെത്രിൻ എന്ന കീടനാശിനിയുടെ അപചയത്തിനായി ഒരു മൈക്രോബയൽ കൺസോർഷ്യം (PDMC1-Pesticide Degrading Microbial Consortium) സൃഷ്ടിക്കപ്പെട്ടു, കൂടാതെ MSM-ൽ കീടനാശിനിയുടെ 91% നശീകരണവും കാണപ്പെടുന്നു. DRNB1-Stenotrophomonas maltophilia, SFN1-Stenotrophomonas [സ്യൂഡോമോണസ്] geniculate, FXE1-Pseudomonas aeruginosa എന്നിവയാണ് കൺസോർഷ്യം ഉണ്ടാക്കുന്ന മൂന്ന് ബാക്ടീരിയൽ ഒറ്റപ്പെടുത്തലുകൾ.

LIST OF ABBREVIATIONS

ABBREVIATIONS	EXPLANATION
DDT	Dichloro diphenyl trichloroethane
OCP	Organochlorine pesticides
OCC	Organic chlorine chemicals
OPs	organophosphates
AChE	acetylcholinesterase
IMS	Intermediate syndrome
OPIDP	Organophosphate-induced chronic neurotoxicity
OPICN	Organophosphate-induced chronic neurotoxicity
CSE	Center for Science and Environment
SPs	Synthetic pyrethroids
NRDC	National research development cooperation
ATSDR	Agency for Toxic Substances and Diseases Registry
nAChRs	Nicotinic acetylcholine receptor
EASAC	European Academics Science Advisory Council
EU	European Union
EPA	Environmental protection agency
LD50	Lethal dose
GCMS	Gas chromatography-mass spectrometry
GC-NPG	Gas chromatography nitrogen phosphorous detector
PTEs	Phosphotriesterases
CBES	Carboxylesterases
OPH	Organophosphate hydrolase enzymes
2-HQ	2-Hydroxyl quinone
FAO	Food and agriculture organization
GST	Glutathione S-transferase
IARC	International Agency for Research on Cancer
FMC	Food Machinery and Chemical Company
LTRES	Life table response experiment
GABA	Gaba aminobutyric acid
ROS	Reactive oxygen species
LDH activity	Lactate dehydrogenase
TMC	Total microbial population
GDH	Glutamate dehydrogenase
LC50	Lethal concentration
TBPC	cyclohexanol
AST	Aspartate aminotransferases
ALT	Alanine aminotransferases
AP	Alkaline phosphatase
MCH	Mean Corpuscular Hemoglobin
NADH	Nicotinamide adenine dinucleotide
METI	Mitochondrial Complex-I e-transport Inhibitors
IRAC	Insecticide Resistance Action Committee
IGR	Insect growth Regulator

TMX	Thiamethoxam
PGPR	Plant Growth Promoting Rhizosphere
DHEA	Dehydroepiandrosterone
HepG2	Hepatoblastoma cell line
THA	Thiamethoxam
PCB	Polychlorinated biphenyl's
PAHs	Polycyclic aromatic hydrocarbons
POPs	Persistent Organic Pollutants

1.INTRODUCTION

The introduction of synthetic chemicals into the agricultural sector was encouraged in order to achieve sustainable agricultural output. Demand for agricultural food supply increased as the world's population increased. To maintain a balance between the supply and demand of agricultural products, the country and the world place an emphasis on agricultural productivity and quality. One of the leading issues encountered in agriculture sector is associated with crop losses due to increasing pest infestation. Crop loss has become a major global concern in the agriculture industry, prompting the widespread use of various agrochemicals for sustainable farming practices. India has an agrarian economy, and despite several past initiatives for favourable agricultural techniques, there has been a significant revolution in the use of chemical pesticides. Pesticide residues build up in the environment because of heavy pesticide application. Applying pesticides to agricultural land has caused 30–40% of the soil to degrade beyond recovery and has an impact on the microbial community's sustainability. accumulated pesticide residues directly contribute to soil deterioration by affecting the physical, biological, and chemical structure of the soil as well as the population of beneficial soil microflora, and indirectly influence the amount of nutrients that are accessible in the soil. Thus, one of the main factors converting fertile soils to non-fertile soils is the accumulation of pesticides. Increased research is being conducted globally on pesticide degradation in an effort to provide environmentally acceptable solutions to the growing problem of soil contamination and degradation.

1.1 Human Population, Food Production, and Crop Management

The human population on Earth has increased by more than a factor of two since 1960. The current population of 7.6 billion people is expected to expand by 70 million people annually, reaching 9.8 billion by 2050, a 30% increase. Due to changes in dietary trends towards high-quality food in developing countries, such as increased intake of meat and dairy products and increased use of grains for livestock feed, it is predicted that this higher population density will result in a 70% increase in demand for food production (FAO, 2009). Most of this population expansion will take place in developing nations. There is a strong need to curb the starvation of billions of humans. According to the Food and Agriculture Organisation of the United Nations (FAO), increases in yields and the number of times crops can be grown on the same land each year are expected to account for 80% of the increase in food production

required in developing countries to keep up with population growth. An increase in an agricultural area is only anticipated to contribute 20% of the additional food production (Fitton *et al.,* 2019).

In the last 50 years, the cumulative effects of the Green Revolution have allowed for a doubling of global food production. The safekeeping and protection of crops, soil fertility, improvement in agricultural productivity and conservation of crops are an integral part of prevailing agriculture practices. This naturally led to the use of pesticides which are considered the most effective and accepted means of plant protection against pests. The usage of crop-production products increases food grain production from 3 trillion to 4 trillion. To feed the 7.8 billion people in the world, agricultural use of antibiotics, water, pesticides, and fertilizer will require significant growth for both crop and animal products. The production of food is highly impacted by pesticides. They might enhance the number of times each year a crop can be planted on the same piece of land while also protecting or increasing yields. This is crucial in nations where there are food shortages.

Agriculture production faces a significant challenge in reducing the existing output losses brought on by pests, diseases and weeds (Popp *et al*., 2013). Pests are any living things that pose a threat to humans, plants and other creatures. Insect pests are the major constraints that cause damaging effects on the productivity of agricultural crops. A vital amount of crop about 17.5%, is damaged due to the non-usage of pesticides, which accounts for INR 90000 Cr per annum (Koli and Bhardwaj, 2018). The appropriate use of crop protection products can increase agricultural production. Pesticides are compounds that are either employed to eradicate or stop the spread of pests. Pesticides can reduce significant crop losses; thus, they will continue to be used in agriculture. Regardless of the economic situation of a country, the use of pesticides to produce food, both for the local population and for export, should adhere to sound agricultural practices. To protect their crops, farmers should only use the bare minimum of pesticides. Pests may have destroyed 70% of crop production if pesticides had not been used (Oerke, 2006).

1.2 Pesticides: Worldwide production and usage in India

World Health Organisation (WHO) defines a pesticide as a chemical compound that is being used to control the population of insects, pests, and rodents and to keep plant diseases under check (Ballantyne and Marrs, 2004). FAO defines Pesticide is any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal diseases, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feeds, stuff or substance that may be administrated to animals for the control of insects, arachnids or other pests in or on their bodies (Marrazza 2014). Pesticides are frequently referred to as “super chemicals”, or “agrochemicals” employed globally to meet the increasing food demand of the growing population. Pesticides differ in their chemical and physical properties from one class to another (El Nemr *et al*., 2012).

A fifteen-to-twenty-fold increase in the number of pesticides used globally serves as an example of a remarkable increase in crop protection intensity (Oerke, 2006). Since the early 1960s, the use of pesticides has drastically expanded; at the same time, the key sources of human nutrition—wheat, rice, and maize—have seen their average yields more than double. Food prices would rise, and production of food would decline in the absence of pesticides. Undoubtedly, the revolution in the production of various agrochemicals has increased yield in agricultural products and protected crops from pests and illnesses. Pesticides currently play a major role in increasing the output, thereby leading to financial benefits for farmers. Crop yield has increased, and disease resistance has improved with the introduction of agrochemicals. Growth in the global population in the 20th century would not have been conceivable without a corresponding rise in food production. Pesticide use determines the production of almost one-third of agricultural products. Fruit production would decrease by 78%, vegetable production by 54%, and cereal production by 32% without the usage of pesticides. A wide variety of pesticides must be used for successful pest management to combat pests and boost agricultural output, as pest infestations account for about 45% of the yearly food production loss [Abhilash and Singh, 2009]. Therefore,

pesticides are essential for decreasing disease and raising crop yields all around the world.

The pesticide industry experienced phenomenal expansion after World War II because of the rising global demand for productive crops and food. Pesticide usage patterns vary depending on the crop, location, climate, and user requirements. Pesticide consumption and usage patterns differ in each country according to their agricultural land and yield type (Kumar *et al.*, 2017). Globally, about 3 x 10^9 kg of pesticides are applied annually with a market consumption of 40 billion dollars each year. In the 2003-2004 time period, the production volume of pesticides was 69,000 MT, and it increased steadily to 217,000 MT in 2018-2019. Approximately, 2 million metric tons of pesticides are marketed annually worldwide (CSA 2009). These 2 million tons of pesticides consist of 47.5% of herbicides, 29.5% of insecticides, 17.5% of fungicides, and 5.5% of other pesticides (Khaled *et* al., 2004; De *et al.*, 2014). In 2019, there were roughly 4.19 million metric tonnes of pesticides consumed worldwide, with China being by far the largest consumer (1.76 million metric tonnes), followed by the United States (408 thousand tonnes), Brazil (377 thousand tonnes), and Argentina (204 thousand tonnes) (Rios -Fuster *et al.*, 2021).

India is the fourth largest worldwide producer and consumer of pesticides, following the US, Japan, and China (Devi *et al.,* 2017), with an annual production of 90,000 tonnes (Khan *et al.*, 2010; Kumaraswamy, 2012). It is the second-largest manufacturer of general pesticides in Asia (Gupta and Agarwal, 2004). The production of pesticides started in 1952 in India, with the formulation of BHC (Benzene Hexachloride), followed by Dichloro-diphenyl-trichloroethane (DDT) near Calcutta. At the outset of the green revolution in 1961, pesticides were massively used to achieve high agricultural production. They have huge economic importance and have a market rate of billions of dollars. A profit of 84.5 billion has been gained in 2019 and it is expected to reach the mark of 130.7 billion dollars by 2023. In India, insecticides account for most of the total production of pesticides. At present, 293 pesticides have been registered in India, and 104 are used regardless of whether they have been banned or restricted worldwide. In India, pesticides are used mostly in paddy fields (26-28%) followed by cotton fields (18-20%). There was a steady increase in pesticide consumption before 2008-09, but it remains consistent in recent

years with an annual consumption of 50,000 MT. The total pesticide production in the country has decreased hardly since 2020.

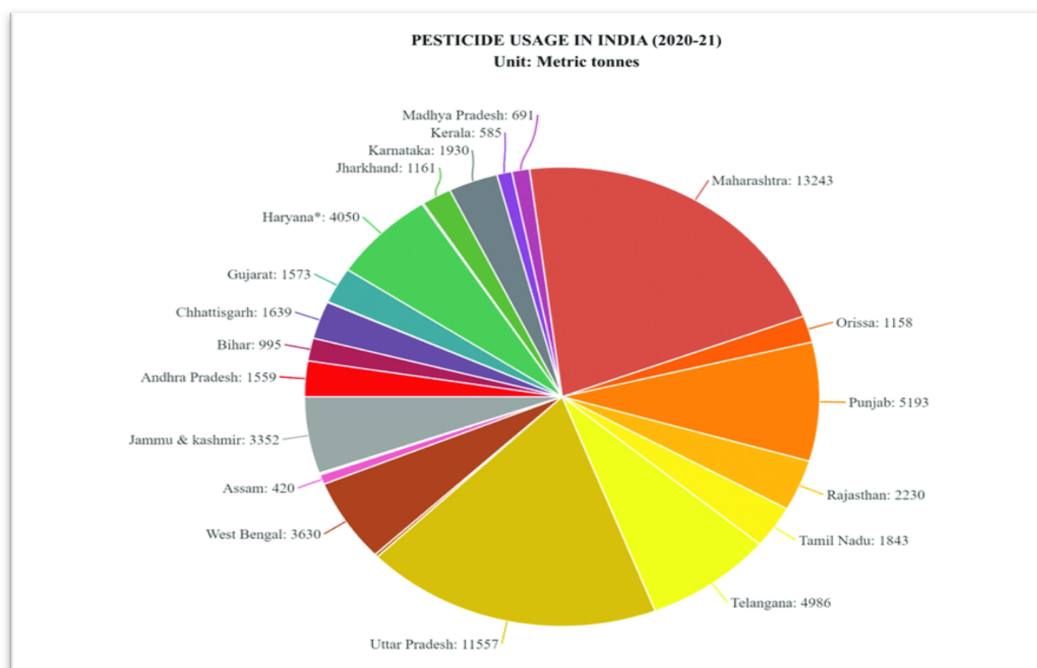


Figure 1.1. Diagram showing Pesticide usage in India (Raj *et al*., 2021)

1.3 Impact of pesticides on the environment

The primary causes of rising pesticide use are population growth and climate change. Although pesticide use helps increase crop yields and the production of nutritious, reasonably priced food, it also has several detrimental consequences on the environment and human health. Such chemical residues affect human health by contaminating the environment and food. Moreover, environmental degradation is a perilous consequence of pesticide contamination dispersed from the target plants. Only 1 to 10% of the pesticides applied reach the target pest, while the remaining spills out into the environment. Pesticides can spread by several routes that include air, wind, water, runoff, or leaching, as well as from plants, animals, and people.

Pesticides have been crucial in the growth and processing of food since pests, insects, illnesses, and weeds can drastically lower the yield of crops. The hazards connected with pesticide use are ignored in favour of using them to preserve food and boost agricultural productivity.The quantity and quality of pesticides used grow consistently and change day by day. The persistent use of pesticides or synthetic chemicals led to

the finding that most of the pests have become resistant to pesticides, which in turn led to a higher usage of the same (Aktar *et al.,* 2009; Ortiz Hernandez *et al*., 2011). Many pesticides were applied extensively without determining their abiding nature and toxic effects on the environment (Berdowski *et al.,*1997). They become potentially hazardous to the environment by direct contact with accidental spills, runoff from application in agricultural areas, and discharge from containers and waste disposal systems.

Environmental pollution has paralleled a growth in industrialization and urbanization due to the uncontrolled use of chemical pesticides. Pesticides used carelessly cause ecological imbalance. The pesticides or chemicals applied in agricultural fields are subjected to a lot of transformations. They can vary or change very dramatically to dangerous or non-toxic substances under natural conditions even in a small area. They find their way into the soil by spray drift, absorbed by plants, leached to groundwater, and degraded into chemical forms. These toxic and non-biodegradable substances accumulate in the food chain and water bodies, and lead to considerable health hazards to all living organisms. The use of pesticides contaminates water resources like surface water and groundwater. The misutilization of pesticides is dangerous for people, livestock, other living organisms, and the atmosphere (Yadav *et al*.,2015). An economic survey done in 2015-16 reported that the use of pesticides without proper guidelines led to an increase in pesticide residues in both the environment and food products (Srijita, 2015).

According to USEPA reports, 60% of herbicides, 90% of fungicides, and 30% of insecticides are identified as potentially carcinogenic (Grube *et al.,* 2011). The uncontrolled use of pesticides in recent decades has contaminated land and water (Rani *et al*., 2008; Heath *et al*., 2010). Further, the use of pesticides to eradicate soil pests also results in the accumulation of chemical residues and their metabolites, which are harmful to the microbial flora of the soil. According to Yates *et al*., (2011), 6.3% of volatile environmental contaminants come from insecticides. Pesticides accumulate in living tissues as a result of their bioaccumulation potential.

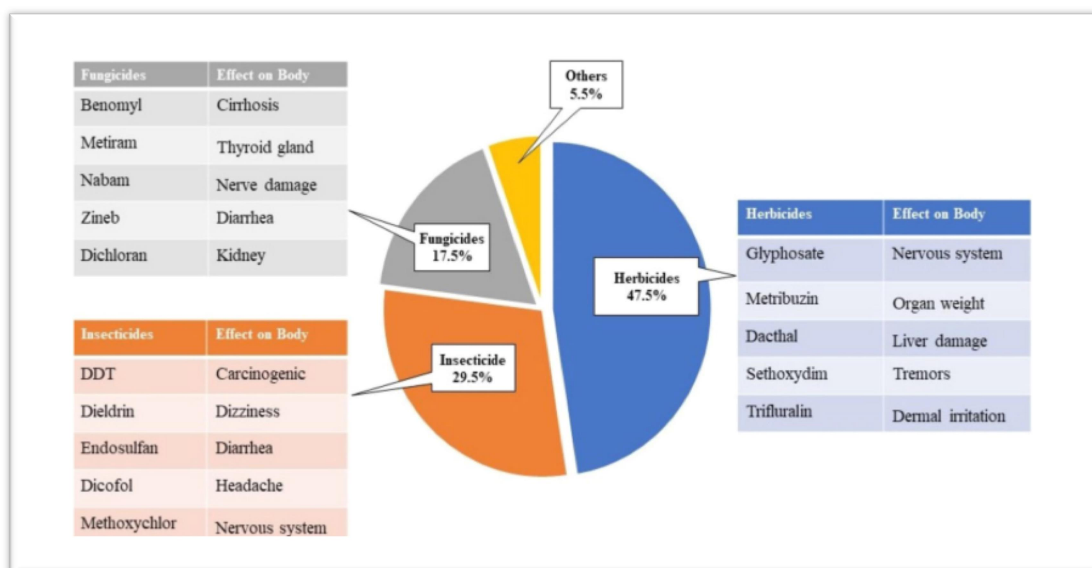


Figure 1.2: Percentage distribution of pesticides and their effect on the body (Nicolopoulou-Stamati *et al*., 2016; Alengebawy *et al*., 2021; Pathak *et al*., 2022)

Pesticides disturb predator-prey interactions and damage biodiversity. Around 33 countries, that is nearly 90% of the globe, use at least one of the hazardous pesticides. Nearly 18 countries use highly hazardous or extremely hazardous pesticides (Tariq *et al*., 2007; El Nemr *et al*., 2012). About 10,00,000 human beings are affected by acute poisoning through contact with pesticides and, a death rate between 0.4 and 1.9% is recorded every year (Qiu *et al*., 2017; Eddleston, 2020; Jia *et al*., 2020). Nearly 70% of mortalities are due to work-related contact with pesticides. Continued exposure to low dosages of pesticides result in the formation of numerous tumours and nervous system disorders (Owens *et al.,* 2010; Bertero *et al*., 2020). Occupational exposure comprises 60 to 70% of pesticide poisonings (Damalas and Eleftherohorinos, 2011).

The two main mechanisms of pesticide contamination of living organisms are biomagnification and bioconcentration (Liu *et al*., 2018; Zhen *et al*., 2019). Bioconcentration is the transmission of a chemical into an organism from the surrounding medium, for example, DDT. The quantity of pesticide residues has been detected as high in fruits and vegetables. The consumption of these foods leads to testicular dysfunction, human infertility, and neurological and gastrointestinal disorders. Oral and respiratory exposure to pesticides causes asthma and respiratory disorders, cancer, diabetes, and Parkinson’s disease (Campo *et al.,* 2013; Asghar *et al*., 2016; Martin *et al*., 2018).

Pesticides enter the food chain and affect non-target species including humans, flora and fauna, and soil enzyme activity (Rani and Sud, 2015; Degeronimo, 2015; Lozowicka *et al*., 2016). It reaches the non-target vegetation and organisms directly or can drift or volatilize from the applied site. The impact of pesticides is related to "the range of oncological (cancer), hematological morbidity, and pulmonary dysfunction, in addition to immune system deficiencies and inborn deformities" . It is important to recall the disastrous history of DDT and the fact that it requires 80 years for semi-decomposition in nature (Wang *et al*., 2010). About 80 to 90% of applied pesticides spread to the non-target vegetation and organisms. Pesticides pollute groundwater through leaching (Rosenbom *et al*., 2015). Pesticide compound changes to residues known as transformation products with a persistence of more than a decade. About 80% of pesticides can be detected, whereas 47% of transformation products are detected in the topsoil (Erinle *et al*., 2016).

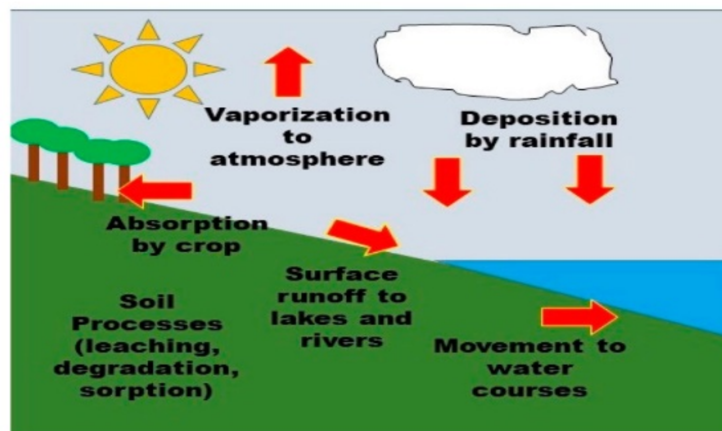


Figure 1.3: Pesticide behavior in the Natural Environment (Tudi *et al*.,2021)

The majority (98%) of pesticide residues reach the soil, and water resources through leaching, sorption, and surface runoff.

1.4 Pesticide Behaviour in Soil

Soils are the naturally occurring physical covering of the earth's surface and represent the interface of three material states: solids (geological and dead biological materials), liquids (water), and gases (air in soil pores). Each soil is a unique product of the combination of geological parent material, glacial and geomorphological history, the presence and activity of biota, and the history of land use and disturbance regimes It is

a mixture of minerals, organic matter, gases, liquids, and countless organisms that together support life on Earth.

A mass of mineral particles alone does not constitute fertile soil. Fertile soils are influenced, modified, and supplemented by living organisms. Plants and animals aid in the development of soil through the addition of organic matter. Soil ecosystem is an intricate form of billions of bacteria, protozoa, complex fungal webs and plants, nematodes, and microarthropods (Yadav and Devi, 2017). It is a dynamic system in which continuous interaction takes place between minerals, organic matter, and living organisms that influences its physicochemical and biological properties. The physical and chemical conditions of soil are influenced by the organic inputs from litter and roots due to the growth of vegetation. It is a vibrant living system consisting of diverse micro and macro fauna and flora.

The soil flora and fauna play a crucial role in countless soil biological functions, along with nitrogen transformation, organic matter decomposition, nutrient release availability, as well as stabilization of soil structure and soil fertility (Khan and Sculion, 2000). The topsoil and subsoil regions are the platforms of the greatest activity of soil flora and fauna. It is also the scaffold for the interaction of pesticide residue with soil flora and fauna (Sarkar *et al*., 2009). Soil is vital for the existence of many forms of life that have evolved on our planet. It is the foundation of all terrestrial ecosystems and is home to numerous living creatures. The rich and diversified community found in soil is home to a wide range of microorganisms.

The large-scale use of pesticides in agroindustry and drastic changes due to urbanization cause a negative impact on nature, which in turn alters the balance of microbial community in the soil. The changes in the environment and the discrepancies of nature pose additional challenges to agricultural soil. The soil used for agriculture is subjected to a variety of conditions that not only affect its physical composition but also its microbiota and related processes (Gupta *et al*., 2008; Erktan *et al*., 2020). Large-scale applications of organic agricultural pollutants like pesticides have both short- and long-term effects on agricultural soils. These pesticides, which are classified as organic pollutants, not only build up in the soil and make it hazardous, but they also make the soil more saline. These environmental factors thrust the local microbial community under duress. Long-term exposure of the soil

microflora to a certain stress situation can aid in the acclimatization of microorganisms to the condition that may necessitate a molecular alteration in organisms. Microbial species can become lethal when exposed to toxic organic pollutants like pesticides (Vischetti *et al*., 2020). A reduced variety of beneficial soil microbes has also been linked to pesticide toxicity (Karpouzas and Singh, 2006).

Pesticides have the potency to leach from soil and contaminate groundwater (Di *et al*.,1998). They could persist and accumulate on the topsoil and become toxic to microorganisms, plants, wildlife, and human beings (Amakiri, 1982; Ayansina and Oso, 2006). The soil stability is affected by the intensity, dissipation rate, and persistent nature of the parent pesticide compound and its metabolites (Margni *et al*., 2002). The persistence of pesticides in the soil is affected by soil humidity, temperature, sunlight, plants, and physiological differences. (Arias *et al*., 2008).

Pesticides can dissipate and degrade into the environment when they are applied to an area or plant using local microbial strains and physicochemical parameters. After entering the ecosystem, they break down into new chemical entities called metabolites, which, depending on their chemical makeup, regulate the persistence of pesticides in soil. These metabolites can be harmful, or non-toxic, and it also introduces the idea of the environmental half-life of pesticides (Liu *et al*., 2015; Valcke *et al*., 2017).

As the primary reservoir of environmental pesticides, soil exhibits a high retention capacity of pesticides in its structures through adsorption. However, it also releases old organic pollutants into the atmosphere, groundwater, and living things as secondary sources. A feature or quality of soil is its ability to filter, break down, and detoxify pesticides. The uptake of pesticides, their toxic kinetics, dispersion, metabolism, and excretion, all have an impact on species. The bioavailability of pesticides in the food chain also has an impact.

Pesticides linger in the soil for a very long time and harm the soil and ecosystem because of the attraction between soil particles and pesticides in sorption systems (attraction is determined by soil organic matter and soil structure). Although pesticides were created to help protect the globe, their overuse has turned them into a necessary evil. Since pesticides are resilient in the environment, they disrupt

important ecological components. Overuse of pesticides disturbs major ecological components. Therefore, it is imperative to decrease or eliminate contaminants from the environment (Paul *et al.,* 2005).

1.5 Soil Microbes: Diverse biotic communities in the soil

A significant class of microbes that maintains the health and productivity of the soil is bacteria. Bacteria are tiny one-celled organisms that are typically one micrometre (4/100,000 of an inch) wide. In general, a teaspoon of fertile soil holds one hundred million to one billion bacteria. In other words, one cubic centimetre of soil can be home to more than one million bacteria (Dick, 2009). Soil microorganisms are the substantial and most diverse biotic community in soil. Soil organisms, especially microbiota, play an essential role in the stabilization of soil structure and fertility of the soil by regulating nutrient dynamics, acting as a nutrient pool, energy transfer through the soil food web, and as sensitive indicators of soil fertility (Kandeler *et al.*, 1996; Wardle *et al*., 2004). Many biogeochemical cycles involve soil microbes. They enhance the health of plants and soil fertility, and by secreting growth regulators, encourage plant development and increase plant production (Nadeem *et al*., 2020). They promote the growth of plant biomass. Moreover, roots interact with soil microorganisms affecting plant growth. Plants rely on a diversity of soil microbes to convert air nitrogen into nitrates, which they may absorb.

The activity and species composition of microbes are broadly influenced by many environmental factors like edaphic properties of soil, i.e., soil modulators, and soil resources. Soil modulators are soil temperature, pH, water potential, and salinity. Carbon and nitrogen are the soil resources that microorganisms compete for. Microbes in the soil are related to above-ground vegetation through the medium of soil (Zartman *et al.,* 2003; Wardle *et al*., 2004). The soil microbial communities are mostly regulated by the diversity and composition of vegetation. Changes in ecosystems, including changes in vegetation and soil conditions, have an impact on soil microbial biomass, phylogenetic diversity, and physiological activities. Soil microbial biomass gives early cues to gauge the stability of the situation, resilience, and hardiness of the ecosystems.

Populations of helpful soil microorganisms may drop because of heavy pesticide application to the soil. Soil scientists claims that if both bacteria and fungi are lost, the soil will deteriorate. The misuse of chemical fertilizers and pesticides has similar consequences on soil organisms as the overuse of antibiotics in people. The indiscriminate use of chemicals may be effective for a while, but eventually, there will not be enough beneficial soil organisms to hold onto the nutrients (Savonen, 1997). Common landscape herbicides interfere with this process. Triclopyr prevents soil bacteria from converting ammonia to nitrite (Hallin and Pell, 1998); glyphosate inhibits the growth and activity of free-living nitrogen-fixing bacteria in soil (Santos and Flores, 1995); and 2,4-D inhibits nitrogen fixation by bacteria that reside on the roots of bean plants (Arias and de Peretti, 1993). The contamination of soil, particularly farmland soil, with pesticides, and the quality of crops and food safety are interwoven. Contamination of soil adversely affects soil microbial population, bacterial diversity, nitrogen transformation, soil animals, and soil enzymes (Feld *et al*., 2015; Sharma *et al*., 2016; Wang *et al.,* 2016).

1.6 Role of microbes in pesticide degradation

Degradation is the process by which a pesticide is broken down after usage. Pesticides are degraded by bacteria, chemical processes, or light after being administered to the target organism. The degradation process may take hours, days, or even years depending on the environmental factors and chemical properties of the pesticide.

There are three different types of pesticide breakdown, microbial degradation, chemical degradation, and photo degradation. Pesticides are broken down by microorganisms like fungi and bacteria, which is known as microbial degradation. Chemical reactions in the soil can break down pesticides. This is known as chemical deterioration. Temperature, pH levels, moisture content, and pesticide binding to the soil all affect how quickly and the type of chemicals that degrade. Pesticides are subject to photo-degradation when exposed to sunlight. All insecticides have some photo-degradation potential, and the rate of degradation is influenced by the intensity of light, exposure time, and the individual characteristics of the insecticide.

Various physiochemical methods have been advocated to reduce pesticide residues in the environment. Despite being hazardous and expensive, physical and chemical

removal of pesticides releases more poisonous chemicals. According to Niti *et al*., (2013), over 6106 synthetic chemicals are generated a year, with even more in the pipeline. While landfilling, incineration, and chemical decomposition are traditional methods that are thought to be effective, they also come with some drawbacks, including complexity and poor public awareness, which can result in significant human loss or worker health issues. By disrupting important life-sustaining processes and upsetting the ecological balance, environmental decontamination can have negative consequences on ecology, including microorganisms, aerial and terrestrial environments, and microbes themselves (Batayneh, 2012).

An innovative way to reduce pesticide contamination sustainably for long-term environmental benefit is pesticide biodegradation. Biodegradation is the process of recycling waste material by converting organic material into nutrients through the biological action of live microorganisms. This process involves the total mineralization of complex chemical molecules into simpler forms such as CO_2, NO_3, H_2O, and other inorganic compounds. The International Union of Pure and Applied Chemistry defined the term biodegradation as the 'breakdown of a substance catalyzed by enzymes in vitro or in vivo'. This process transforms the toxic organic chemicals into less toxic or non-toxic substances. Microorganisms eliminate hazardous toxins to preserve a sustainable environment with a healthy and productive ecosystem (Desisa *et al*., 2022). Pesticides degrade in soil mainly through mineralization, and the main pathway for mineralization is microbial metabolism and demolition (Hassan and Metwally, 2013). Microorganisms are recognized for their impact and numerous applications in promoting human welfare and play a vital part in the breakdown of pesticides. Microbial degradation is either catabolic or co-metabolic. It increases the fertility of agricultural soil.

The two major biological mechanisms that lead to pesticide degradation are microbiological reactions in soil and water and pesticide metabolism which organisms consume as part of their absorption of food. The energy consumption through the metabolism of pesticides is not completely utilized for the body functions and it severely disrupts the reproduction and growth of the organism (Yang and Zhang, 2019; Ouyang *et al*., 2020; Pathak *et al.,* 2020). Since pesticides are introduced directly to agricultural fields, it has been discovered that soil-dwelling

microorganisms develop tolerance to pesticide stress conditions. Microbes develop capacities for pesticide metabolism and degradation as a means of adaptation in pesticide-challenged environments.

The biodegradation process is influenced by biotic (microbial agent, competition, growth kinetics, inoculum density) and abiotic (pH, temperature, inoculum preparation: moisture, nutrients availability) factors (Zhu and Selim, 2002). The selection of resistant and suitable/competent microbial strains is one of the most important steps in successful biodegradation. As a result, several studies (Boivin *et al*., 2005; Arora *et al*., 2019) have been conducted to examine the ability of naturally occurring microorganisms to degrade pesticide-contaminated soil. Such investigations are carried out using a sequential method, which entails the identification and characterization of pesticide-degrading microorganisms, the examination of certain degradation features in microbes, and the measurement of the actual reduction in pesticide concentration under research.

One of the most efficient approaches for degrading environmental toxins is bioremediation; it makes use of bacteria as a potential tool. It is the process by which organic wastes are biologically degraded under controlled circumstances to a safe state or concentrations below the set limits of the regulatory bodies. Bioremediation is the safe elimination method of contaminates from a polluted area. It is an economically efficient, eco-friendly technique that avoids additional environmental damage. Due to their eco-friendliness and successful use in many nations, bioremediation technology can successfully clean up pesticides (Mohamed, 2009). Numerous in-situ initiatives, such as bioventing, biosparging, and bioaugmentation, as well as ex-situ devices, such as land farming, biopiling, and bioreactors, are included in the field of bioremediation. It has also been used to detoxify pesticides using phytoremediation techniques including phytodegradation (phytotransformation), phytovolatilization, rhizoremediation, etc. Phytoremediation, microalgae bioremediation, myco-remediation, and bacterial pesticide degradation are only a few of the environmentally friendly pesticide remediation methods used today (Abhilash and Singh, 2009).

According to Singh (2008), high pH and inoculum density (CFU/mL) are crucial criteria for degradation and in-situ bioremediation. Techniques based on

biodegradation are becoming more and more common for ecological restoration. Using inoculants isolated from contaminated environments (where contamination has occurred over the years), several researchers (Talwar *et al*., 2014; Hegde *et al*., 2017; Khajezadeh *et al*., 2020; Chen *et al*., 2022) may have accomplished highly successful eradication of contaminants. These natural microbes provide a dual benefit of detoxifying the pollution first, and secondly not endangering other local species of flora and fauna. The process of biodegradation is greatly influenced by a variety of biotic and abiotic variables.

Microbial systems are primarily used to mediate the biodegradation of pesticides. A certain class of enzymes that can be produced by microbes can catalyze the breakdown of pesticides from contaminated areas. The removal of pesticide residues from the soil and water environment was found to be accomplished with both pure cultures and mixed cultures of bacteria and fungi. The chemical structure is the first thing that microorganisms break down, turning it into inorganic parts that they can then absorb. To improve the biodegradability of the pesticide, advanced techniques like bioaugmentation, bio-stimulation, and natural attenuation are used. These techniques include adding powerful bacteria, more nutrients, and native species to the contaminated area. The main bacterial genus responsible for removing pesticides from polluted settings includes *Pseudomonas, Azotobacter*, *Flavobacterium*, and *Arthrobacter*. Microbes consume the pesticide as food, produce H_2O and CO_2, and weaken their negative effects on the environment. Such pesticides build up in the soil system and serve as carbon and electron sources for soil microorganisms. Environmental parameters (such as temperature, pH, moisture, nutrients, and water availability), pesticide exposure length and concentration, bacterial type, and growth factors are all crucial for effective biodegradation. The process of pesticide breakdown can be impacted by several variables, including the microbial culture, cultivation method, inoculum size, growth under elevated pesticide percentages, adaptation, rhizosphere interactions, and reaction to environmental influences (Conde-Avila *et al*., 2021). According to Bhatt *et al*., (2021), the production of biomolecules by bacteria, such as biosurfactants, bioemulsions, and biofilm, is the major indicator of their capacity for biodegradation. By enhancing the bioavailability of these biomolecules in soil for microbial breakdown, the production of these biomolecules facilitates the process of complicated pollutant degradation.

Bacteria with novel or advanced features can be employed to break down pesticides. One of the reasons bioremediations have not fully achieved commercialization is the fact that there has only been limited research in bacterial populations (Holt *et al*., 2010).

Microbial degradation is regarded as an effective, economical, and environmentally acceptable approach for eliminating harmful pesticides from the environment. In this context, this study proposes to determine the effectiveness of native soil bacteria in the metabolism and investigate the degradation of the most frequently and heavily used commercial-grade pesticides. It also focuses on the utilization of microbial consortia to clean up specific pesticide-contaminated samples. The site for the proposed study is the tea plantations in Anaimalai in South India.

1.7 Significance of the study

Soil is the most complex and vibrant living system of all microbial habitats. The soil microbial community plays a major role in the stabilization of soil structure (Sarkar *et al*., 2009). The continuous use of pesticides, compost, and the application of several physical and chemical pesticide removal techniques alter the soil parameters and the structure of soil microbial communities. Biodegradation, particularly microbial degradation, is an efficient technique for the degradation of pesticides, and has been explored by several scholars focusing on different types of ecosystems (Johnsen *et al*., 2005; Verma *et al.,* 2014; Moorman, 2018). Almost 65% increase in the number of publications related to pesticide degradation studies is evident proving its importance in the scientific fraternity (Sundari *et al*., 2019). This thesis explores microbe assisted degradation of different pesticides. This study explores the biodegradation ability of the indigenous bacteria in the tea plantation soils of Anaimalai in the Indian state of Tamil Nadu. It is primarily concerned with the isolation of pesticide-degrading microorganisms from the environment for biodegradation applications.

The Valparai plateau, which is dominated by tea, coffee, and cardamom plantations is surrounded by the Anamalai Tiger Reserve and other protected forests and wildlife sanctuaries. It is a human-modified biodiversity hotspot in the tropics (Cincotta *et al*., 2000). Land-use modification in the form of tea and coffee plantations dates back to more than a hundred years at Valparai (Muthiah, 1993). The study acquires great

significance considering the status of the region as earmarked as a major area of biodiversity and protected under the Government as a buffer zone. Traditionally this region had been a land of plantations and is a major source of livelihood of the people residing there. It has been reported that there is widespread use of pesticides in the region to protect the crops from pests and to increase productivity (Daniels, 2003). As a result, the rate of persistence of pesticides is bound to increase in the soil and will gradually alter the soil structure and stability of the microbial community. The investigation on pesticide breakdown by soil bacteria in tea plantations (especially tea rhizosphere bacteria) of Anaimali Hills was carried out by Sarkar *et al*. in 2010. The pesticides previously investigated were old-generation pesticides and most of them were replaced with new-generation pesticides. Sarkar *et al*., (2010) examined the degradation of pesticides dicofol, propargite, and propiconazole by bacteria isolated from tea rhizosphere. They also studied the biodegradation of heavy metals by rhizosphere bacteria. However, such studies were conducted more than a decade ago and were based on the old generation pesticides. There is an urgent need to make a pesticide degradation study on Anaimali Hills to explore the condition of the soil and the result of the application of new pesticides to maintain the stability of the environment.

The present study mainly focuses on the microbial degradation of pesticides Deltamethrin, Quinalphos, Ethion, Propargite, Thiamethoxam, Glyphosate, Fenpyroximate, and Spiromesifen along with the parameter optimization to understand the optimum temperature, pH, and cell concentration for the maximum level of degradation of pesticides by bacteria. It helps to conduct ex-situ bioremediation more efficiently and productively. The biosurfactant and biofilm producing ability of microorganisms are studied at a preliminary level in the present study. The present investigation also focuses on the development of microbial consortia for pesticide degradation. Microbial consortia are a group of diverse microorganisms that can act together in a community. Microbial consortia improve the biodegradation process by reducing the half-life persistence of the pesticides (Jabeen *et al*., 2015; Ahmad *et al*., 2018). It also improves soil productivity by enhancing plant growth. All these earlier studies (Foster, 2004; Sarkar *et al*., 2009), highlighted the biodegradation of pesticides by soil bacteria. The present study aims to full fill the void in the earlier studies, and as well as the findings of the present

study may substantiate valuable insights to the ongoing research works related to pesticide biodegradation.

1.8 Hypothesis

The observations of previous related research and a pilot study conducted in the region provided insight for the current study and its relevance. It is hypothesized that "the use of specific bacterial isolates from tea garden soil, that possess the ability to break down pesticides, is the optimal method for the biodegradation of pesticides from classes including Organophosphates, pyrethroids, neonicotinoids and tetronic acid derivatives in polluted sites, while also ensuring minimal environmental consequences". The study intends to address the problem of eradicating pesticide residue from contaminated sites.

The investigation includes an assessment of pesticide tolerance of specific indigenous bacterial isolates found in tea garden soil. These isolates will be subjected to various pesticides commonly used in tea and coffee plantations in India, including quinalphos, deltamethrin, thiamethoxam, ethion, propargite, glyphosate, spiromesifen, and fenpyroximate. Soil samples from tea plantations will be collected and analysed to identify and study the bacteria with the ability to degrade pesticides. Additionally, a bacterial consortium will be developed for the enhanced degradation of pesticides.

1.9 Thesis objectives

The primary goal of the study is to identify and analyse the potent indigenous bacterial species found in tea plantation soils, which are capable of degrading persisting pesticide residues that are used in tea plantations. The study specifically examines eight distinct pesticides, quinalphos, deltamethrin, thiamethoxam, ethion, propargite, glyphosate, spiromesifen, and fenpyroximate which are predominantly used in Indian agricultural fields and tea plantations. It also seeks to optimise the factors that lead to the maximum level of pesticide degradation. It intends to explore the biosurfactant and biofilm characteristics of the bacterial isolates at the preliminary level. The study examines the efficacy of the bacterial consortia in degrading pesticides in liquid MSM.

In other words, the objectives may be listed and defined as given below:

1. To determine the quantity of pesticide residue, present in the soil samples.
2. To determine the soil bacterial population in both plantation and forest areas through comparative analysis.
3. To study the physiochemical parameters (pH, Electrical conductivity, Moisture content) and nutrient contents (Organic carbon, Total N, P, K, Micronutrients) of the soil samples.
4. To isolate and characterize the soil bacteria that possess the ability to degrade organophosphate, neonicotinoid, and pyrethroid pesticides in the sampling sites.
5. To prepare a combined microbial or bacterial consortium that degrades selected pesticides.

1.10 Literature survey and Gaps addressed in the present work.

The investigation on pesticide breakdown by soil bacteria in tea plantations (especially tea rhizosphere bacteria) of Anaimali Hills was carried out by Sarkar *et al.* in 2010. The pesticides previously investigated were old-generation pesticides and most of them were replaced with new-generation pesticides. Sarkar *et al.*, (2010) examined the degradation of pesticides dicofol, propargite, and propiconazole by bacteria isolated from tea rhizosphere. They also studied the biodegradation of heavy metals by rhizosphere bacteria. However, such studies were conducted more than a decade ago and were based on the old generation pesticides.

The pesticides chosen are Deltamethrin, Quinalphos, Ethion, Propargite, Thiamethoxam, Glyphosate, Fenpyroximate, and Spiromesifen. Among the selected pesticides studies on microbial degradation of Fenpyroximate and Spiromesifen are limited.

Scientific studies on biodegradation of ethion by soil bacteria were not carried out after 2004 (Foster, 2004). The biodegradation studies of propargite were done by Sarkar *et al.,* in 2010. These two pesticides are often used commonly in agricultural fields and particularly in plantation sites. Therefore, the biodegradation studies of these two pesticides are crucial to maintaining soil structure and stability of microbial communities.

Parameter optimization studies (Bhattacharjee *et al*., 2014; Ambreen *et al*., 2020; Varghese *et al*., 2021) help to understand the optimum temperature, pH, and cell concentration for the maximum level of degradation of pesticides by bacteria. The optimizations studies of these pesticides by bacteria is very limited. It helps to conduct ex-situ bioremediation more efficiently and productively.

1.11 Structure of thesis

The thesis is structured into six chapters that includes the introduction and conclusion as the first and fifth chapters. This is followed by a segment on recommendations. The second chapter discusses the literature review of environmental pollution, chemical properties of pesticides and their toxicity, biodegradation of pesticides, and microbial consortia studies for pesticide degradation. The third chapter deals with the materials and methods chosen for the study. The fourth chapter consists of results and discussion sessions.

Table No : 1.1 Pesticides selected for the present study.

SL.NO	PESTICIDE	PESTICIDE TYPE	SUB GROUP PRESENT	PESTS CONTROLLED
1.	Deltamethrin	Insecticide	Pyrethroid	Spiders,Aphids, Bird mites
2.	Ethion + Cypermethrin	Insecticide	Organophosphate	Red spidermite
3.	Fenpyroximate	Acaricide	Pyrazolium	Phytophagous mites
4.	Glyphosate	Herbicide	Organophosphate	Annual & perennial herbs
5.	Propargite	Acaricide	Sulfite ester	Spider mites
6.	Quinalphos	Insecticide	Organophosphate	Mites, bollworms
7.	Spiromesifen	Insecticide	Tetronic acid derivative	Spidermite, grass mite
8.	Thiamethoxam	Insecticide	Neonicotinoid	Aphids, Ants

2. REVIEW OF LITERATURE

2.1 Pesticides

Pesticides are a broad spectrum of chemical or biological substances designed to kill or retard the growth of pests that damage or interfere with the growth of shrubs, trees, timber, and other vegetation desired by humans. Pesticides are necessary for economical pest management (Gouma, 2009). They are used for plant crop protection against pests (Ozkara *et al*., 2016; Sun *et al*., 2018).

Pesticides are used as an old practice and have been used in agriculture since early 1900. In 1000 BC, the Chinese used Sulphur as a fumigant. Japanese used a mixture of whale oil and vinegar as a pesticide in the 16th century. In the 17th and 19th centuries, tobacco leaves, and different plant and flower extracts were used as insecticides. At the beginning of the 20th century, 10% sulphuric acid is used as a pesticide. The development and use of pesticides increased dramatically during the post-II world war. About 140 tons of pesticides are employed at the beginning of 1940. The commonly used organic pesticides are plant extracts and heavy metals as inorganic chemicals. During the 1940s the use and production of synthetic pesticides increased (Gavrilescu, 2005).

In 1991, 23,400 pesticide products are registered with the US Environmental Protection Agency (Singhvi *et al.,*1994; Frazar, 2000). Among six hundred thousand tons of pesticides used in 1997, 77% constitute for agriculture, commercial, and government purposes, 12% for private use, and 11% are used in other sectors (Fishel, 2005). Agriculture holds the largest share in the use of pesticides. The primary source of pesticides in the ecosystem is agriculture and forestry.

2.2 Worldwide usage of pesticides

Pesticides are used in around two million tons and over five hundred compounds are registered as pesticides or metabolites of pesticides per year worldwide, with China being the largest contributor, followed by US and Argentina, which is fast expanding. It is predicted that global pesticide use will climb to 3.5 million tons by 2020 (Parte *et al*., 2017; Sharma *et al*., 2019).

Agriculture constitutes 85% of pesticide usage and public health uses about 15% of pesticides (Kim *et al*., 2011). The total global output of food grains has risen from five hundred million tons to seven hundred million tons since the beginning of the

20th century. According to the USEPA, more than 1180 pesticides have been registered globally, of which 435 are herbicides, 335 are insecticides and 410 are fungicides. Since 1950, the use of pesticides has surged up to fifty times worldwide, with 2.5 million tons being used annually (Mahmoud and Loutfy, 2012).

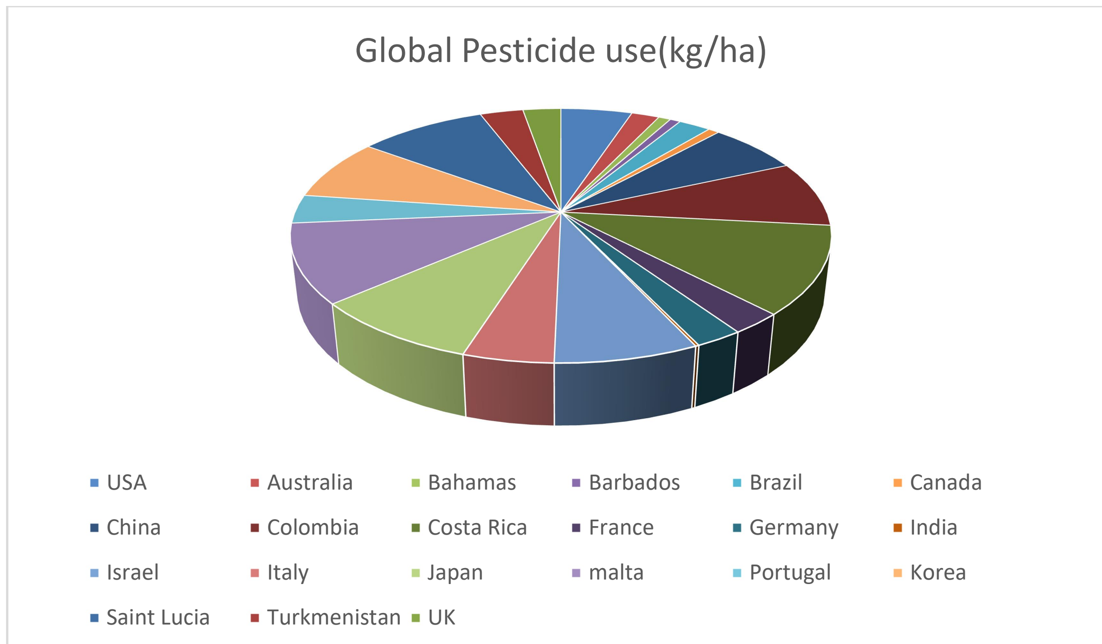


Figure 2.1: Global Pesticides usage (http://www.fao.org/faostat/en/#data/EP/visualize)

2.2.1 Pesticide Usage in India

Pesticide use in India is at 0.5 kg/hectare, with organochlorine pesticides accounting for the majority of this (Abhilash and Singh, 2008; Bhat and Padmaja, 2014). Pesticide production in India began in 1952 with the production of benzene hexachloride followed by DDT. Pesticide synthesis is grown dramatically. India produced about five thousand metric tons of pesticides in 1958, which climbed to 85,000 metric tons in the mid – 1990's with the registration of 145 chemicals, India is the second largest manufacturer of general pesticides in Asia (Gupta, 2004) with insecticides being the most common pesticides produced. The annual pesticide consumption of India is about 56,120 tons. In India, pesticides are purchased for crops like cotton, paddy, rice, and vegetables (Sarkar *et al*., 2009).

Pesticide production in India is dominated by insecticides followed by fungicides and herbicides. In our country, pesticides are mainly used to control insect pests (62.23%). About 19.16% are used as fungicides, 14.4% as herbicides, and 4.2% are used as others. Herbicide consumption is very low in India because control of weed is mainly

done by hand. The total consumption of pesticides in India from 2005 to 2010 was 210,600 metric tonnes as per official data of the Directorate of Plant Protection, Quarantine, and Storage, Govt. of India (Verma and Saxena, 2014). Moreover, 59670 metric tonnes (MTs) of total pesticide (technical grade) consumption were reported in the year 2018-19, across the country.

Nearly 9000 species of insects and mites, 50000 species of plant pathogens, and 8000 species of weeds damage crops globally. In India, an average of 45% of crop loss occurs annually due to pest infestation, and 35% is lost during storage (Abhilash and Singh, 2008). India produces an average of 250 million tonnes of grains annually, but it also losses 11-15% of that amount because of pests and other factors (Walter *et al.*, 2016). Insecticides lowered crop loss due to pests from 42% to 35% (Pimentel, 2009). The cultivation of agricultural products requires pesticides and the production of about one-third of agricultural products involves the use of pesticides.

The insecticide act of 1968, as revised on August 20th, 2014, section 9(3), states that 246 pesticides have been registered for use in India. Insecticides, herbicides, fungicides, and other pesticides are applied at the rates of 65%, 16%, 15%, and 14% respectively. Throughout the world, herbicide consumption was higher than that of insecticides, fungicides, and other pesticides, however, in India, insecticide use has exceeded the herbicide consumption. This might be attributed to an increase in insect pests' attacks brought on by the subtropical regions' characteristic warm and humid climates (Odukkathil and Vasudevan, 2015). Crop production needs to be expanded to keep up with the rising demand in developing nations like India, where agriculture accounts for a large portion of the gross domestic production (GDP). Despite using far fewer pesticides on average than wealthy nations, India has a considerably greater problem with pesticide residue. As a result, cases of pesticide contamination are increasing constantly. About 50% of vegetables were found to be contaminated by various pesticides, of which, 16% were above Maximum Residue Level (MRL) (Mohamed *et al.*, 2014).

2.3 Classification of pesticides

WHO classified pesticides into four major groups based on their toxicity level and lethal dose (LD 50) has been set as a benchmark. The four different classes are: -

- Class I – extremely hazardous
- Class II – highly hazardous
- Class III – moderately hazardous
- Class IV – slightly hazardous

Drum (1980) recommended three approaches for the classification of pesticides. The three methods are: -

- Chemical structure of the pesticides.
- The mode of entry of the pesticides.
- The action of pesticides and the organism they kill.

Chemical pesticides are classified based on their structure as carbamates, organophosphates, organochlorines, and pyrethroids.

The mode of entry is the method used by pesticides to interact with or reach the target pests (Gerolt, 1969). Based on entry modes pesticides are classified into systemic pesticides, irregular pesticides, and physical pesticides. In systemic ones, they act on the untreated part of the organisms, and they act on target pests in the case of irregular pesticides. In physical pesticides, they encounter pests through the lesions. The route of exposure involves gastric toxins, contact exposure, expectorant, and evaporators.

Table 2.1 Target organism: This classification includes chemicals, biopesticides, and antimicrobials.

Target Pest	Pesticide Type
Algae	Algicides
Birds	Avicides
Bacteria	Bactericides
Fungi and Oomycetes	Fungicides
Insects	Insecticides
Viruses	Virucides
Rodents	Rodenticides
Miticides/Acaricide	Mites
Molluscicides	Snails
Nematicides	Nematode

Table 2.2 The classification of pesticides based on the Chemical Structure.

Sl. No	**Category of pesticide**	**Mode of Action**	**Members**
1.	Organophosphate	Inhibition of the enzyme acetylcholinesterase (AChE)	Malathion Parathion Chlorpyrifos Methyl parathion
2.	Organochlorine	A) Affects the parasympathetic nervous system by hyperexcitation of nerves, caused by leakage of sodium ions. B) Affects the central nervous system by hyperexcitation of nerves as the organochlorine binds to the GABA receptor and inhibits chlorine flow into the nerves.	DDT Endrin Endosulfan Dieldrin Aldrin Pentachlorophenol
3.	Carbamate	Inhibition of the enzyme acetylcholinesterase (AChE)	Carbofuran Carbaryl Aldicarb Phenoxycarb
4.	Neonicotinoid	Affects the central nervous system of the insects, causes excitation the nerves, which leads to paralysis and finally death	Imidacloprid Acetamprid Thiamethoxam Thiacloprid
5.	Anthranillic diamide	Works specifically on the insect ryanodine receptors	Dinotefuran Chlorantriniliprole (Coragen) Cyantraniliprole
6.	Synthetic pyrethroid	Prevents repolarization of the axonic membrane thereby permanently paralyzing the insect.	Allenthrin, Bifenthrin, Cypermethrin, Cyphenothrin

2.3.1 Other classes of pesticides

Triazines, such as atrazine, simazine, and ametryn, are another class of chemical pesticides linked to reproductive harm and endocrine disruption (Mnif *et al*., 2011; Kniewald *et al*., 2000; Jin *et al*., 2014). Furthermore, a probable statistical link between triazine herbicides and breast cancer incidence was discovered (Kettles *et al*., 1997). The most well- known is atrazine, a widely used herbicide that has been linked to oxidative stress (Jin *et al*., 2014), cytotoxicity (Liu *et al*., 2015; Ma *et al*., 2016), and dopaminergic effects (Huang *et al*., 2014). Furthermore, atrazine exposure in experimental animals has been linked to reproductive damage (Song *et al*., 2014), and sexual maturation delays (Breckenridge *et al*., 2016).

Synthetic pyrethroids, such as fenvalerate, permethrin, and sumithrin, are among the safer insecticides currently available for agricultural and public health purposes (Kolaczinski and Curtis, 2004, Bhattacharya *et al*., 2006). However, there is evidence for their ability to display endocrine-disrupting activity (Garey and Wolff, 1998; Pandey *et al*., 2006; Hassin and Metwally, 2013), and to affect reproductive parameters in experimental animals including reproductive behavior (Moore *et al*., 2001; Jaensoson *et al*., 2006). Furthermore, a recent study related more than one pyrethroid metabolite to DNA damage in human sperm, raising concerns about possible negative effects on human reproductive health (Jurewicz *et al*., 2015). It should also be mentioned that there are also concerns about their ability to display developmental neurotoxicity (Shafer *et al*., 2005; Ahmad *et al*., 2015).

There are certain restraints for conventional insecticides such as their persistent nature, toxicity to the environment, and low dissipation (Table 2.3). As a result, distinct types of insecticides that are synthetic analogs of natural products, and bacterial fermentation products are introduced. They are: -

1. Neonicotinoids
2. Bacterial fermentation products
    - Spinosyns
    - Avermectins
    - Milbemycins
3. Phenyl pyrazoles
    - Fipronil

4. Oxadiazines
    - Indoxacarb
5. Anthranilic diamide
6. Pyridine azomethines
7. Pyridine carboxamide
8. Benzoyl phenyl ureas
9. Triazapentadiene.

Table 2.3 Limitations of conventional insecticides.

Organochlorines	They are highly persistent, residual problem, and toxic to humans
Organophosphates	Resistance, resurgence, and toxic to environment
Carbamates	Toxic to the natural environment
Synthetic pyrethroids	Resistance, resurgence, and induces secondary outbreak.

2.3.2 Organochlorine Pesticides

Organochlorine compounds or pesticides (OCP) contains carbon, hydrogen, and chlorine in their chemical composition. They are also known as chlorinated hydrocarbons, chlorinated organics, chlorinated insecticides, and chlorinated synthetics. They are the acute neurotoxic chemicals used in agriculture, public health, and forestry in the late 1940's. In public health sector it controlled several vector-borne diseases, like yellow fever, malaria, and typhus (Bus and Leber 2001; Li *et al*., 2007). The common physical properties are low water solubility and high lipid solubility. The presence of chlorine atom in their structure makes them moderately soluble and semi-volatile (Shen *et al*., 2005). Diphenyl aliphatic, which include DDT, DDD, dicofol, ethylene, chlorobenzilate and methoxychlor are the earliest group of organochlorines. The DDT was discovered and used in 1939, by Paul Hermann as an efficient insecticide (Matolcsy *et al*., 1988). The organochlorine pesticides are categorized based on toxic action, mechanism, and specific symptomology. The two main groups are DDT-type compounds and chlorinated alicyclices. The most prevalent compounds of organochlorine groups that are detected in the environment are DDT and HCH, because of their wide application in agriculture and public health. These compounds are recalcitrant in nature and resistant to biodegradation (Parte *et*

al., 2017). In India, the consumption rate of DDT was 21,642 tons in the period from 2000 to 2006. India, DDT was found in the soils collected from Goa and along the coastal regions of Kolkata in the range of 0.4 to 124ng/g (Chakraborty *et al*., 2015). The Stockholm Convention on Persistence Organic Pollutants (SCPOPs) held in 2001, detected 12 POPs as "dirty dozen", including OCP such as DDT, HCH, alderin, dieldrin, endrin, chlordane, hexachlorobenzene, mirex, heptachlor, and toxaphene (Syed *et al*., 2014), and other two chemicals are polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). During the fourth meeting of the Stockholm Convention on POPs in 2009, another nine industrial chemicals were declared as "nasty nine".

2.3.2.1 Mode of action and degradation of Organochlorine pesticides

It prevents the proper nerve impulse transmission in insects and animals, by disrupting the balance of sodium and potassium ions within the axons of neurons. The damaged neurons will result in muscle twitching, convulsions, and death. The compounds toxicity is affected by substituents attached to parent molecule, symmetry of chemical structure, solubility, sorption etc. (Kaushik and Kaushik, 2007). The potency of DDT is negatively correlated with temperature that is it becomes more hazardous when temperature drops. The chemical nature of OCPs is different, like some OCP compounds are unstable, while others adhere to soil and atmospheric particulate matter. The OCP was banned and restricted in the years between 2001 and 2010.

These compounds accumulate in the environment and enter food chains and remain constant for decades in the soil. The two major degradation pathways of OCCs are reductive dichlorination under anaerobic conditions, and dehydrochlorination occurs aerobically. Bacteria comprise the major group of microorganisms that degrades and metabolizes the OCCs. The soil biota belonging to genera *Bacillus, Pseudomonas, Arthrobacter,* and *Micrococcus* are the potential degraders of these compounds (Braund *et al*., 1971). The fungi like *Penicillium miczynskii*, *Aspergillus sydowii*, *Trichoderma sp., Penicillium raistrickii*, and *Bionectria sp.,* were isolated with the potency to degrade OCCs (Parte *et al*., 2017). The endosulfan is degraded by *P. aeruginosa* (Jayashree and Vasudevan, 2007), *S. maltophilia* and *Rhodococcus erythropolis* (Kumar *et al*., 2008), *Achromobacter xylosoxidans* (Li *et al*., 2009),

Arthrobacter sp., KW (Weir *et al*., 2013), *Citrobacter amolonaticus* G4, and *Acinetobacter lowffii G5* (Ozdal *et al*., 2016). The microbial degradation of OCCs is enhanced by the addition of biochar to the contaminated site (Gregory *et al*., 2015).

2.3.3 Organophosphate Pesticides / Compounds

Synthetic organophosphorus compounds are used as pesticides, plasticizers, air-fuel ingredients, and chemical warfare agents (Singh and Walker, 2006). Organophosphate pesticides are second-generation pesticides introduced in the 1960s and are ecological alternatives to organochlorine pesticides (Jaga and Dharmani, 2003). It is discovered by German Chemists in 1983. They are defined as the degradable organic compounds and derivatives of phosphoric, phosphonic, phosphinic, or triphosphoric acids that are usually in the form of esters, amides, or thiols and with a central phosphorous atom (Balali, 2014). Organic phosphate pesticides are commonly coupled with two organic groups and an additional side chain consisting of cyanide, thiocyanate, or phenoxy groups. Organophosphate pesticides are the most commercially favored group of pesticides and are applied globally due to their potent insecticidal and low persistence nature. They are used for the protection of crops and livestock and in the control of vector-transmitted diseases (Yasmeen *et al*., 2009). The global consumption of organophosphate pesticides accounts for about 38-40% of all the pesticides applied worldwide (Singh and Walker, 2006). Several study reports state that the consumption of organophosphate pesticides increased by 61% (4.30 billion USD to 7.06 billion USD) in 2017 (Sundari *et al*., 2019). India shares top rank with countries like the USA, China, Ukraine, and Iran in the consumption of organophosphate pesticides Table 2.4 (Sundari *et al*., 2019).

Table 2.4 Global annual Organophosphate pesticide (OPP) consumption (Sundari *et al*., 2019)

Average annual consumption (Tonnes)	**Countries**
0-50	Austria, Nigeria, Finland, Iceland, Ireland, Hungry, Slovakia, Tajikistan
50-300	Germany, Greece, Netherlands, United Kingdom, South Sudan, Zimbabwe, Nepal, Sri Lanka, Panama
300-1000	Poland, France, Portugal, Italy, Bangladesh, Sudan, Costa Rica
1000-3000	Argentina, Spain, Myanmar, Thailand, Malaysia, Japan
3000-20000	India, the United States of America, China, Ukraine, Iran

The use of organophosphate compounds started in the early 1800s. Two scientists, Lange in Berlin and Schrader at Bayer AG, Germany discovered and studied the use of organophosphates as insecticides. The German military terminated the usage of OPP as an insecticide. After the second world war, organophosphorus groups of pesticides were reintroduced as organophosphate esters or nerve gas. In earlier times OPPs were known as organic phosphates, phosphorous insecticides, nerve gas relatives, and phosphoric acid esters. Tetraethyl pyrophosphate was the first developed organophosphorus insecticide, in 1937 by Dr. Gerhard Schroder in Farbenfabriken bayer laboratories in Germany (Dragun *et al.*, 1984) and the usage of OPP was uplifted due to their low persistence rate. By the end of World War II different other organophosphate insecticides with high insecticidal activity like ethyl and methyl parathion were introduced by Bayer, ICI groups. The examples of OPPs are given in Table no 2.5. The remarkable usage of OPP started with 12,000,000 kg in 1992 to 32,500,000 kg in 2014.

Table 2.5: Examples of OPPs based on their chemical structure and usage.

Sl. No	Examples of OPPs	Type (Based of chemical structure)	Type (Based on use)
1.	Tabun, sarin, Soman		Nerve agents
2.	Dichlorvos	Phosphates	Insecticide
3.	Mevinphos	Phosphates	Insecticide
4.	Chlorpyrifos	Thiophosphates	Insecticide
5.	Acephate	Phosphoramids	Insecticide
6.	Malathion	Dithiophosphate	Insecticide
7.	Dimethoate	Dithiophosphates	Insecticide
8.	Phorate	Dithiophosphates	Insecticide
9.	Fenamiphos	Phosphoramides	Insecticide
10.	Isothirophoate		Ophthalmic agent
11.	Trichlorofon	Phosphonic ester	Antihelmintics
12.	Tributos		Herbicide
13.	Tricresyl phosphate	Phosphates	Plasticizer (Industrial agent)

2.3.3.1 Structure, Classification, and Mode of Action

Organophosphate pesticides are esters of phosphoric acid with a central phosphorous atom. They are put together in different combinations of carbon, nitrogen, oxygen, and sulphur. The structure of OPP is the phosphoester linkage and it determines the mode of action of these pesticides. The basic structure of OPP is depicted in figure 2.2 where R1 and R2 represent different aryl or alkyl groups. These groups are bonded to phosphorus (P) through oxygen (O) or Sulphur (S) atom. The R1 and R2 groups are linked directly to phosphorus and an oxygen or sulfur atom respectively in most of the OPPs and in some of them, these groups were linked with mono or di-substituted amino groups. The X group is also known as the leaving group and this group can belong to aromatic, aliphatic or heterocyclic groups. The X group is released from the phosphorous upon hydrolysis of the ester bond. Organophosphates are classified based on their chemical structure. The linkage of R1 and R2 groups with P through various atoms leads to the formation of various compounds. It includes: - phosphates (Linked through O2), phosphonates, phosphinates, phosphorothioates, phosphonothioates, phosphorodithioates, phosphorotrithioates, and phosphoroamidothioates (C atom bonded with P through NH) (Gupta, 2006). They are also classified into reversible and non-reversible acetylcholinesteresas inhibitors based on their mode of action (Jokanovic and Stojiljkovic, 2006). Examples of non-reversible inhibitors are tabun and sarin, warfare agents. OPs are also generally divided into aliphatic, phenyl, and heterocyclic derivatives.

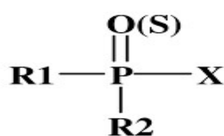


Figure 2.2 Basic structure of Organophosphate Pesticide.

Phosphates	Thionate	Phosphorodithioate	Phosphorothiolate	Phosphoramides
$R^1O-P(=O)(-OR^3)-R^2O$	$R_1O\ OR_2\ P(=S)----OR_3$	$R_1O\ OR_2\ P(=S)----SR_3$	$R_1O\ OR_2\ P(=O)----SR_3$	$H_2N-P(=O)(-NH_2)-NH_2$
• Monocrotophos • Dichlorovos • Mevinphos	• MethylParathion • Fenthion • Chloropyrifos	• Dimethoate • Phorate • Malathion	• Amethoate • Vamidthion • Demeton-s-methyl	• Acephate • Fenamiphos • Methamidophos

Figure 2.3 Classification of OPPs based on their structure (Sundari *et al*., 2019)

Organophosphate pesticides are neurotoxic compounds, and they irreversibly inhibit the acetylcholinesterase enzyme (Singh and Walker, 2006). It binds with the cholinesterase enzyme and deactivates the enzyme activity by irreversible phosphorylation in target pets. This enzyme plays a pivotal role in the nervous system by regulating the levels of acetylcholine molecules and managing the transmission of nerve impulses in the region of the brain and skeletal muscles. The inhibition of acetylcholinesterase results in the subsequent synaptic junction accumulation of acetylcholine, which builds up the continuous stimulation of postsynaptic tissues (Guimaraes *et al.*, 2011). The rate and degree of acetylcholine inhibition depend on the structure of OPP, the nature of their metabolites, and other factors such as pH and temperature (Worek *et al.*, 1999). The OPP interacts with AChE and forms AChE-complex. The acetylcholinesterase is reactivated rapidly after inhibition, depending on the chemical structure of the organophosphate used. The reactivation of the enzyme varies upon exposure to the type of OPP and the chemical group that is attached to the active enzyme site. Organophosphate pesticides also inhibit other esterases like butyl cholinesterase and neuropathy target esterase in the target pest. It also induces nicotinic and muscarinic effects in the peripheral and central nervous system.

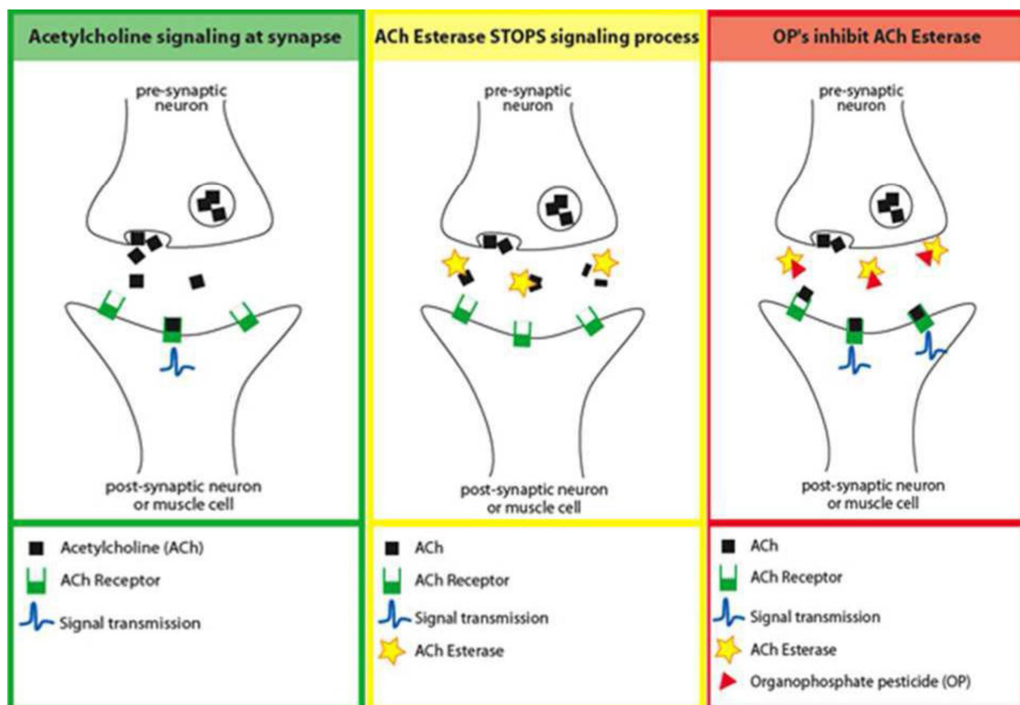


Figure 2.4 Mode of action of OPP (Prins *et al.*, 2014).

2.3.3.2 Toxicity of Organophosphate Pesticides

Organophosphate pesticides are more susceptible to human consumption because they are highly soluble in water. These compounds were the commonly detected pesticides in polluted areas (Jaramillo *et al*., 2016). There are two types of OPP toxicity, acute and chronic OP toxicity. Acute OP toxicity can be expressed in three different levels, they are cholinergic syndrome, intermediate syndrome (IMS) and delayed neuropathy. The inhibition of acetylcholinesterase results in the overstimulation of postsynaptic muscarinic and nicotinic receptors. This causes cholinergic syndrome. The intermediate syndrome was first described by Senanayake and Karalliedde (1987). It is so termed because it starts around 24-96 hours after initial exposure and could last up to 5 to 18 days and up to 210 days in rare cases (Das *et al*., 2013). Acute toxicity of OPP primarily affects the parasympathetic, sympathetic, and central nervous systems. Jokanovic and Skrbic (2012) defined IMS as either a decline in the number of functional cholinergic receptors at the post-junctional membrane or a failure in the release of acetylcholine. It manifests between 10 to 20 days after a single exposure to OPP (Jokanovic and Skrbic, 2012) and it is a very rare type of acute toxicity. OPIDP causes paralysis about two or more weeks after OPP exposure. The mechanism of OPIDP is that it interrupts the axon transports as well as the interaction between glial and axon cells. Exposure to large doses of OPP results in acute neural cell death in the brain (Masoud and Sandhir, 2012). OPIDP outbreaks were reported in some countries such as Morocco and the USA (Lotti and Moretto, 2005). It is also observed in animals such as sheep and chickens and in some experimental rodents. Masoud and Sandhir (2012) studied the OPIDP toxicity caused by parathion, leptophos, methamidophos, and malathion. Chronic OP toxicity is of two types, organophosphate-induced chronic neurotoxicity (OPICN) and chronic OP-induced neuropsychiatric disorder or copind. The neurodegeneration resulting from exposure to OPs at small subclinical doses is referred to as OPICN (Abou-Donia, 2003). Some of the signs and symptoms of chronic toxicity are anxiety, apathy, confusion, disorientation, impaired memory, dizziness, insomnia, fatigue, and impaired vigilance. Velmurugan *et al*., (2017) reported the correlation between subclinical doses and diabetes among Indian farmers.

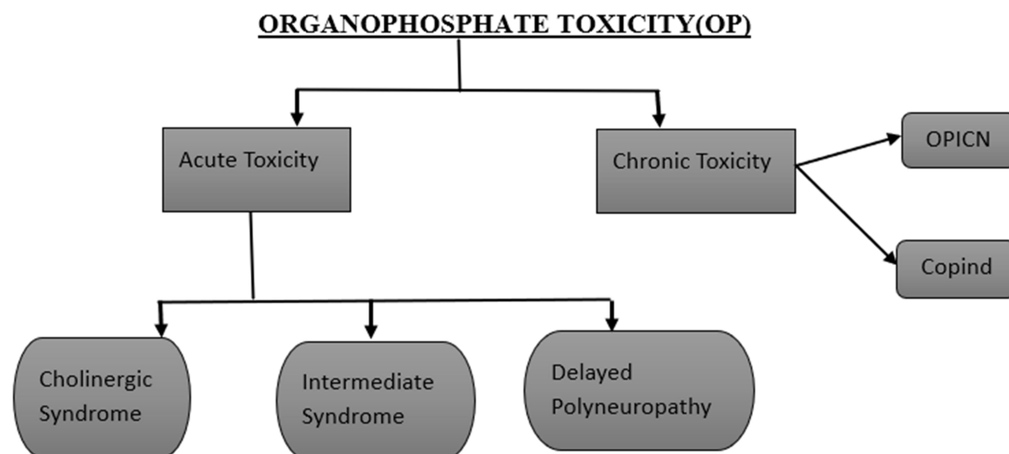


Figure 2.5 Toxicity levels of OPPs.

Soils are contaminated with the intensive use of OPPs. It causes loss of soil fertility, acidification of soil, nitrate leaching, increased resistance of weed species and biodiversity loss (Tilman *et al*., 2002; Mohapatra and Pattanaik, 2012). Han *et al*., (2017) reported the residues of chlorpyrifos in the soil samples of nut fields in China. Masia *et al*., (2015) traced three organophosphate pesticides namely chlorpyrifos, diazinon, and ethion in soil, sediment, and sludge samples from Turia river, Spain. Kumari *et al*., (2008) reported that soil samples of paddy wheat, paddy-cotton, and sugarcane fields of Hisar, Haryana were contaminated with chlorpyrifos, malathion, and quinalphos residues. Bishnu *et al*., (2009) determined residues of ethion and chlorpyrifos in the soil samples taken from West Bengal. Jacob *et al*., (2014) studied the contamination of cardamom field soil samples of Idukki district, Kerala. Contamination of water bodies caused by OPP is also a threat to the deterioration of different environments worldwide. Pujeri *et al*., (2010) reported chlorpyrifos residues in water samples taken from lakes of Bijapur, Karnataka. Similarly, several studies reported the presence of OPP residues in different water bodies (Ahad *et al*., 2000; Bishnu *et al.,* 2009; Ilyas *et al*., 2019) like ground water (Kumari *et al*., 2008) and surface water (Dehghani *et al*., 2012; Lari *et al*., 2014;). The extensive use of OPP also contaminates agricultural products like tea (Kottiappan *et al*., 2013; Greenpeace India report 2014), food grains (Yang *et al*., 2008; Marchis *et al*., 2012), vegetables (Singh and Gupta, 2002; Parveen and Kumar, 2005; Bhanti and Taneja, 2007; Kumari *et al*., 2008; Chuanjiang *et al*., 2010; Ojha *et al*., 2011; Ananda and Somasekhar, 2012; Chandra and Kumar, 2015; Lozowicka *et al*., 2016) and fruits (Hussain *et al*.,2002; Parveen *et al*., 2004; Vemuri *et al*., 2014; Jallow *et al*., 2017). The

contaminates the food products like ghee and butter (Kumari *et al*., 2005), honey (Choudhary and Sharma, 2008) and soft drinks. It caused around 3 million poisonings and 200,000 human deaths annually (Karalliedde and Senanayake, 1988).

Singh *et al*., (2014) stated that the usage of OPPs triggers the inevitable pollution of the environment. The residues of OPP were found worldwide in human blood, urine, fish tissues, breast milk and bovine milk. As per Mathur *et al*., (2005) residues of various OPPs were detected in blood samples of human beings, collected from villages of Punjab. Wang *et al*., (2016) detected OPP residues in urine samples collected from adult farmers in China. The maximum residue level of pesticides was found in fishes and the minimum level in man in the order fish>chick>goat>human (Mehta *et al*., 2008). Srivastava *et al*., (2008) reported the contamination of bovine milk samples with residues of extremely hazardous (class Ia) pesticide, methyl parathion.

2.3.3.3 Degradation pathways of organophosphate pesticides

OPPs are extremely used in agricultural fields, because of their biodegradable nature. The biological and physicochemical degradation of OPPs was intensively studied by several researchers. OPs with C-P linkage are thermally stable and chemically inert and are resistant to photolytic degradation and chemical decomposition (Greaves and Letcher, 2014; O'Brien, 2016).

- Photodegradation

Adequate research was done on the photodegradation of OPPs (Lacorte and Barcelo, 1994; Derbalah *et al*., 2004). Degradation in both the aqueous environment as well as in the gas phase, photolysis plays a crucial role in the OPPs degradation. Lacorte and Barcelo (1994) studied the degradation of OPPs fenitrothion. The photodegradation products range from oxidized P=S bonds to isomerized products of OPPs.

- Hydrolysis

Hydrolysis is the most efficiently studied degradation pathway of OPP. It is the fundamental pathway for the complete degradation of the molecule because it makes the compounds more vulnerable for further degradation. The hydrolysis of OPP includes the cleavage of two bonds, the breaking of the P=S bond in the case of phosphorodithioates and phosphorothioates or the P=O bond in the case of phosphorothioates (Lai *et al*., 1995). In diazinon a phosphorothioate, the hydrolysis

takes place by the cleavage of the P-O bond. In malathion, the most dominant pathway for degradation is alkaline hydrolysis (Wolfe *et al*., 1977; Wang and Hoffman, 1991). Hydrolytic degradation enhances the adsorption process (Smolen and Stone, 1998).

- Enzymatic degradation or enzymatic hydrolysis

Many aquatic species could produce hydrolyzing enzymes for OPPs. Ohshiro *et al*., (1997) reported that the metabolism of naturally occurring organophosphates and halogenated organic compounds resulted in the formation of organophosphorus acid anhydrases. Wang *et al*., (2011) termed these enzymes as organophosphorus acid anhydrases, and they have also been referred to as paraoxonase, esterase, phosphotriesterase, diisopropyl fluorophosphatase, somanase, and parathion hydrolase. The phosphotriesterase activity is the first and most important step in detoxification. This enzyme hydrolyses the OPPs at the central atom of pesticides that is hydrolyzing the phosphorous atom. Liu *et al*., (2008) identified and characterized these enzymes from squid, fish, invertebrates such as Rangia cuneata, protists, and various thermophilic and other bacteria.

- Microbial degradation

Stroud *et al*., (2007) clearly reveal that scientists have been searching for indigenous bacteria, particularly from contaminated areas that can utilize and degrade a wide range of pollutants. Based on the bioremediation potential, the physiology, ecology, and evolution of microbes can be extensively studied. The major two enzymes involved in the degradation of OPP are hydrolase and phosphotriesterase (Liu *et al*., 2001; Zhongli *et al*., 2001; Ortiz-Hernandez *et al*., 2011; Gao *et al*., 2012). Ragnarsdottir (2000) found that microbial degradation is ten times more efficient and faster than photolysis (physical degradation). Singh and Walker (2006) illustrated the principal reactions involved in the degradation process, such as oxidation, hydrolysis, alkylation, and dealkylation. Microorganisms degrade OPPs through the hydrolysis of P-O alkyl and aryl bonds with the help of enzymes such as hydrolase, phosphotriesterase, and carboxylesterases (Bhadbhade *et al*., 2002; Sogorb and Vilanova, 2002; Kapoor and Rajagopal, 2011; Zuo *et al*., 2015). A large group of bacterial genera can degrade OP compounds. The first microorganisms capable to degrade organophosphorus pesticides were isolated and identified in the Philippines in 1973 as *Flavobacterium sp*. ATCC 27551 (Sethunathan and Yoshida, 1973). Mulbry

(2000) isolated two OPP degrading bacterial strains *Pseudomonas diminuta MG* and *Flavobacterium ATCC 27551* and analyzed the presence of OPH enzyme in them.

Scientists isolated a wide range of OPPs-degrading microorganisms like *Aspergillus niger* (Liu *et al*., 2001; Pandey *et al*.,2015), *Aspergillus fumigatus* (Thakur *et al*., 2022), *Cladosporium cladosporoidies* (Gao *et al*., 2012), *Penicillium raistrickii* and *Aspergillus sydowii* (Alvarenga *et al*., 2014), *Scenedesmus, Stichococcus, Chlorella* (Megharaj *et al*., 1987; Caceres *et al*., 2009), *Cyanobacteria, Nostoc* (Megharaj, 1987; Ibrahim *et al*., 2014), *Anabaena* (Ibrahim *et al*., 2014), *Oscillatoria* (Salman *et al*., 2016), *Arthrobacter sp*. (Ohshiro *et al*.,1997) and *Plesiomonas* (Zhongli *et al*., 2001), and *Stenotrophomonas* (Yang *et al.,*2006). Countless studies on OPP biodegradation were done in India and isolated numerous OPP degrading microorganisms such as *Exiguobacterium sp*. And *Rhodococcus sp* (Phugare *et al*.,2012), *Bacillus aryabhattai* (Pailan *et al*.,2015), *Sphingobacterium s*p.(Abraham and Silambarasan, 2013), *Pseudomonas putida, P.aeruginosa, P.stutzeri* and *Klebsiella* (Sasikala *et al*., 2012), *Pseudomonas, bacillus, Agrobacterium* (Madhuri and Rangaswamy, 2009; Maya *et al*., 2011), *Providencia stuartii* (Vijaya *et al*., 2008), *Ralstonia eutropha, E.cloacae* (Bano & Musarrat, 2003; Rani and Juwarkar, 2012), *Staphylococcus, Licheniforms* (Karishma and Sharma, 2014).

2.3.3.3.1 Factors affecting the degradation of OPPs.

The two significant factors that affect the behavior of organophosphates in the environment are the adsorption and mobility of OPPs. The rate of adsorption and degradation is affected by several factors such as solubility, volatility, charge, polarity, molecular structure, and size of the pesticide. The degradation of OPPs can be retarded or enhanced by the process of adsorption by soil particles. Smolen and Stone, (1998) proved that abiotic hydrolytic degradation enhances the process of adsorption and they also stated that the process of adsorption was affected by physicochemical forces like Vander Waal's forces, dipole-dipole interactions, hydrogen bonding, and ion exchange.

2.3.4 Pyrethroids

Synthetic pyrethroids (SPs) are the chemical and photostable analogs of pyrethrins. Pyrethrins are the recognized active insecticide compounds that are present in the flowers of *Chrysanthemum cinerariaefolium*. Pyrethrins are powerful insect

repellents, with low mammalian toxicity, and low persistence and they have a broad spectrum of activity against insects (Rehman *et al.*, 2014). These compounds have never been used for plant protection on a large scale in agriculture because they show rapid degradation in the environment that is the half-life of pyrethrins in sunlight is less than five hours. Pyrethroids are synthesized by the groups Sumitomo Chemical Co. in Japan and NRDC in England (Bradbury and Coats, 1989). There are two generations of synthetic pyrethroids (SPs), which are the results of the modifications of the molecular structure of pyrethrins. The most prominent pyrethrins are pyrethrin I and pyrethrin II. The other four active ingredients of pyrethrins are cinerin I and II and jasmolin I and II. The first generation of synthetic pyrethroids was developed in the late 1960s and consists of pyrethrin derivatives such as bioallethrin, tetramethrin, resmethrin, and bioresmethrin. In the 1970s the first-generation pyrethroids are replaced by second-generation pyrethroids due to their unstable nature in sunlight. The second generation includes permethrin, cypermethrin, and deltamethrin. Later, other insecticides such as fenvalerate, lambda-cyhalothrin and beta-cyfluthrin were synthesized (Kidd & James, 1991; Katsuda, 1999). Pyrethroids are also classified or segregated into Type I (T-syndrome tremors) and Type II (CS syndrome-choreoathetosis with salivation) based on their neurotoxicity levels (Verschoyle and Aldridge, 1980). There could be different binding sites in the brain for the Type I and Type II pyrethroids (Lawrence and Casida, 1982).

2.3.4.1 Chemical structure of pyrethroids

Pyrethrins consist of six insecticidally active lipophilic esters: pyrethrin I and II, jasmolin I and II, and cinerin I and II. A chrysanthemic acid linked to aromatic alcohol through an ester linkage is the basic component of pyrethroids. Pyrethroids are derived by altering the molecular structure of pyrethrins, that is by modifying the chrysanthemic acid moiety of pyrethrin I and esterifying the alcohols.

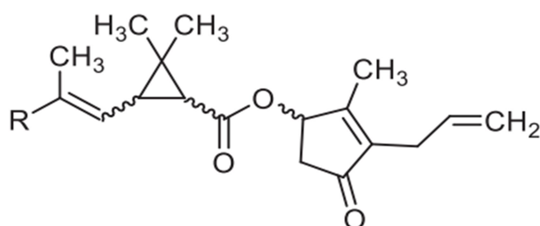


Figure 2.6 General structure of pyrethroids (Coats, 1990)

They differ from other pesticides with the presence of one to three chiral centers. As a result, a pyrethroid compound consists of two to eight isomers and the insecticidal activity varies according to the isomers of pyrethroids. They produce two pairs of diastereoisomers, cis and trans because of two chiral centers in the cyclopropane ring of acids. The main two isomers are 1S which is non-insecticidal and α-R cyano substituted isomers (Schleier and Petrson, 2011). The insecticidally active ones are only those with R configuration at cyclopropane C-1. Alterations in the cyclopropane carboxylic acid moieties increase insecticidal potency and photostability (Soderlund *et al*., 2002). Most of the pyrethroid compounds consist of halogens in their chemical structure, which contributes to their greater persistence and high residual activity. Synthetic pyrethroids retain at least two degradophores for example, carboxy ester, and germinal dimethyl group. The degradation is also depended on the isomer characteristics of the pyrethroids (Stok *et al*., 2004; Liu *et al*., 2005).

2.3.4.2 Classification of pyrethroids

The synthetic pyrethroids are classified into two different classes based on their toxicological and physical properties (cyano group)

Table 2.6 Classification of Pyrethroids based on chemical structure (Laffin *et al*., 2010; Sundari *et al*., 2019;)

Allethrin	Cyhalothrin
Bifenthrin	Cypermethrin
d-phenothrin	Cyfluthrin
Permethrin	Deltamethrin
Resmethrin	Fenvalerate
Tetramethrin	Fluvalinate
Tefluthrin	Lambda-cyhalothrin

The production and applications of pyrethroid insecticides have significantly increased when the use of organophosphate pesticides (OPs) was banned or limited. It is also considered the safer alternative for organophosphate pesticides because of its unique ability to knock down insects at lower application rates, high efficiency, and

low mammalian toxicity. Pyrethroid insecticides have been used to control pests in various sectors for more than 20 years. About one-fourth of the world's pesticide market is contributed by pyrethroid insecticides (Laffin *et al*., 2010; Perez *et al*., 2010; Chen *et al.,* 2011).

The insecticidal property of pyrethrins I first recognized by an American person in the 19th century. In 1949, allethrin, the commercially successful pyrethroid is introduced. Resmethrin is introduced in 1967 and permethrin in 1973, which is 10 to 100 times more stable than resmethrin. Fenvalerate and Pseudo-pyrethroids (non-ester pyrethroid) are introduced in the 1980s and after that cycloprothrin, the hybrid pyrethroid and an analog of DDT is synthesized. In the United States, about 16 pyrethroids are registered for use in a variety of agricultural products (Bryant and Bite, 2003).

Pyrethroids are broad-spectrum pesticides with several advantages, high biological activity, more stability in sunlight, lower water solubility, higher lipophilicity, domain pesticide for malaria control, several agricultural benefits, and are highly effective against a wide range of insects. The insecticidal efficiency of pyrethroids is associated with the ability to induce a toxic effect in the cells of the nervous system of insects (Burr and Ray, 2004). Pyrethroids disrupt the activity of the sodium channels that are responsible for the signal transmission of nerve impulses, by permitting the flux of sodium ions and thus leading to the paralysis and the gradual death of insects (Burr and Ray, 2004; Davies *et al*., 2007; Hintzen *et al*., 2009).

The two factors which determine the toxicity and efficacy of pyrethroids are the stereochemical structure of the insecticide and the geometric or optical isomer of the molecules. Pyrethroids are combined with certain synergists such as piperonyl butoxide, piperonyl sulfoxide, and sesame to enhance the effectiveness of the insecticides. The level of activity of pyrethroids is determined by the penetration of the insecticide into the pest, its metabolism, specificity of the target site, and sensitivity. The pyrethroids are generally safe for mammalian species, but their highly toxic doses cause hypersensitivity to sensory stimuli and a tingling sensation on the skin (Vijverberg and Bercken, 1990). The toxicity of pyrethroids varies with environmental factors like temperature (Sparks *et al*., 1983).

Even if, pyrethroids are thought to be safer than other insecticides, their broad and extensive usage in a vast variety of fields has led to environmental contamination, it also affects non–target organisms like fish and aquatic insects, beetles, bees, parasitic wasps, and microorganisms (Herve, 1985). They are also responsible for disruptions of the endocrine system, suppression of the immune system, reproductive damage, and increased chances of cancer in humans (Zhang *et al*., 2010). Due to its high lipophilic nature, it shows a high affinity to binding to soil and sediment particles. So, it is imperative need to remediate pyrethroid-polluted environments. Natural degradation of pyrethroid occurs in several ways such as photochemical reactions, photo-oxidation, photo-isomerization, ester cleavage, chemical oxidation, biodegradation, and decyanation (Liu *et al*., 2010; Mukherjee *et al*.,2010; Abraham and Silambarasan, 2013). The biological approach, bioremediation which is based on the catabolic activity of pesticide-degrading bacteria, is the most promising and effective remediation method to reduce or minimize the concentrations of pyrethroids in the environment (Tao *et al*., 2012; Ruan *et al*., 2013; Cycon *et al*., 2014; Akbar *et al*., 2015). Microbes play an important role in the degradation of pyrethroids in soil. The degradation of pyrethroids in soil depends on the type of insecticide, soil, climate, the species of microorganism, and microbial population diversity. Biodegradation by microorganisms is considered as the safest, nondisruptive, reliable, and cost-effective method for pesticide degradation. Enzymes play an important role in the microbial degradation of pyrethroids by detoxifying and hydrolyzing the ester bonds in the insecticide. The pyrethroids are primarily degraded by microrganisms by hydrolysis of ester bonds by carboxylesterases which results in the formation of carboxylate and alcohol (Sogorb and Vilanova, 2002; Aranda *et al*., 2014). Numerous pyrethroid-degrading microorganisms are isolated from soils: - *Yersinia frederiksenii* (Lee *et al*., 2009), *Cladosporium sp*. (Chen *et al*., 2011), *Burkholderia picketti* (Zhai *et al*., 2012), and so on.

2.3.5 Neonicotinoid Insecticides

Neonicotinoid insecticides are introduced in the 1990s. They are the derivatives of synthetic nicotinoids and have been applied for the protection of crops from a variety of insects and pests (Simon-Delso *et al*., 2015). They are the most effective pesticides for controlling sucking insect pests, such as aphids, white flies, leaf-plant hoppers, thrips, micro-lepidoptera and coleopteran pests (Jescheke *et al*., 2011). They are also

used in non-agricultural fields such as household sectors, lawn, and garden for controlling termites. Recent studies show that it is used as an ectoparasiticide (Jescheke and Nauen, 2007). Neonicotinoid was developed from the compound, nithiazine, which is like the historical insecticide nicotine. Neonicotinoids are widely used all over the world nearly in 120 countries on 450 crops (Douglas and Tooker, 2015; Simon-Delso *et al*., 2015). It shares nearly one-fourth of market production. Berheim *et al*., (2019) found that they are highly efficient, and have high insecticidal activity at very low doses, and have low toxicity to vertebrates including humans.

Neonicotinoids conquered approximately one-quarter of the pesticide market, and their annual production was about 600,000 tons (Simon -Delso *et al*., 2015). The first neonicotinoid insecticide was imidacloprid developed by Bayer Crop in the mid-1980 and this holds the highest shares in the global pesticide market for many years (Iyyadurai *et al*., 2010). There are seven different neonicotinoid insecticides developed so far (Figure 2.7). Neonicotinoids were classified based on active group present in them, chemical structure, presence of pharmacophore moiety, and development generation (Jescheke and Nauen, 2007).

2.3.5.1 Structure and Mode of Action

Neonicotinoids are systemic, nAChRs agonists and neuroactive insecticides, and it acts on nicotinic acetylcholine receptors (nAChRs). The nAChRs play a key role in the intervention of fast excitatory synaptic transmission in the CNS of insects and it is one of the important molecular target sites of insecticides. They are highly used in a prophylactic fashion that is in seed treatments. They inhibit the nAChRs on the postsynaptic membrane of insect, involves the nicotine acetyl bile, and causes the excitation of associated nerves nearby, thus resulting in the paralysis and death of the target pests (Zhang *et al*., 2018).

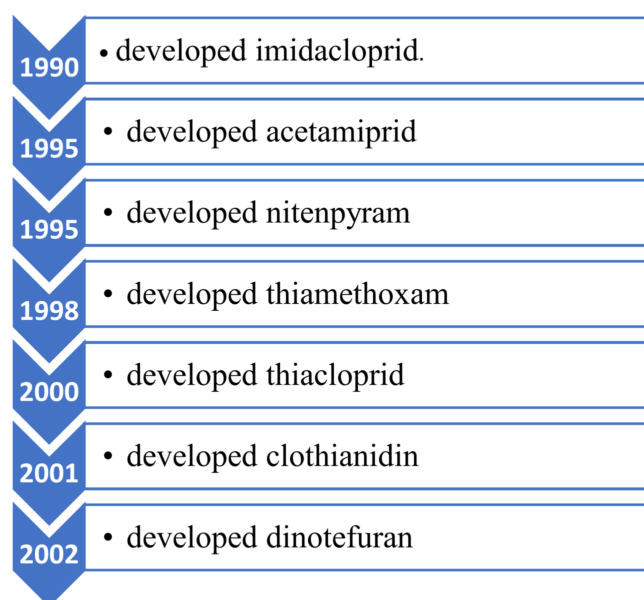


Figure 2.7 Examples of neonicotinoid insecticides.

Table 2.7 Classification of neonicotinoids

	ACTIVE GROUP	PHARMACOPHORE MOIETY	GENERATION	CHEMICAL STRUCTURE
Nicotine, nithiazin, acetylcholine	Nitromethyelene group			
Imidacloprid	Chloropyridinyl group	N-nitroguanidines	First	5-membrane ring
Acetamiprid	Chloropyridinyl group	N-cyanoamidines	First	Non-cyclic
Nitenpyram	Chloropyridinyl group	nitromethylenes	First	Non-cyclic
Thiamethoxam	Chlorothiazolyl group	N-nitroguanidines	Second	6-membrane ring
Thiacloprid	Chloropyridinyl group	N-cyanoamidines	First	5-membrane ring
Clothianidin	Chlorothiazolyl group	N-nitroguanidines	Second	Non-cyclic
Dinotefuran	Tetrahyfrofuran group	N-nitroguanidines	Third	Non-cyclic

They also cause muscle tremors and cell fatigue. Ellis *et al.*, (2017) explained that neonicotinoids were absorbed and circulated throughout the plant system to act against pests. They are taken up by plants and transported to all plant parts including

flowers, pollen, and nectar (Hoppe *et al*., 2015). It shows efficient acropetal translocation in plants. The potency of neonicotinoids is determined by the chemical structure of the overall molecule (Tomizawa and Casida, 2005). The neonicotinoids consist of three structural components: - heterocyclyl methyl moiety, heterocyclic or acyclic spacer, and =NNO2, =CHNO2 or NCN tip.

2.3.5.2 Toxicity of Neonicotinoids

Neonicotinoids are a threat to the environment (Pisa *et al*., 2017; Goulson, 2018; Giorio *et al*., 2021). Only 5% of the active ingredient of neonicotinoids is taken up by the crop plant and the remaining diffuses into the wider environments which results in the contamination of natural sources. Neonicotinoids exposure will occur through different pathways, like through pollen, nectar, guttation fluids, soil, air, and non-crop vegetation (Roubos *et al*., 2014; Mogren and Lundgren, 2016; Calvo-Agudou *et al*., 2019). The widespread use of neonicotinoids raised concerns about the exposure and toxicity effects on non-target organisms (Douglas and Tooker, 2015; Calvo -Agudo *et al*., 2019). The high levels of exposure were reported for different species of farmland birds (Humann-Guilleminot *et al*., 2019) and sublethal impacts on large vertebrates such as deer (Gibbons *et al*., 2015; Berheim *et al*., 2019). The risk of exposure to neonicotinoids is dependent on the rate of application, application type, and crop type. They disrupt both food chains and biogeochemical cycles. Neonicotinoids firmly bind to soil; the retention rate of neonicotinoids is higher in loam and clay soil and the lower retention rate was observed in sandy soils. Among neonicotinoids, Imidacloprid and Clothianidin are extremely persistent with a half-life of 3000 to 6931 days in soil (Van der Sluijs *et al.,* 2013). They are water-soluble compounds, but less persistent in water than in soil (Gibbons *et al.,* 2015), and the major contamination of waterbodies occurs from run-off after acute rainfall, which poses a risk for aquatic organisms (Hladik *et al*., 2014; Main *et al*., 2016). Bonmatin *et al*., (2015) explained that the solubility of neonicotinoids in water depends on the temperature, water pH, and the formation of neonicotinoid application.

Neonicotinoids are detected as a key factor responsible for the decline of global pollinators. They caused mass poisonings of honeybees in Germany and Italy (Bortolotti *et al.,* 2009; Pistorius *et al*., 2015). Residues of neonicotinoids in honeybees were found all over the world (Mitchell *et al.,* 2017). In 2018, the

European Union (EU) prohibited the use of seeds treated with clothianidin, thiamethoxam, and imidacloprid.

2.3.5.3 Degradation of Neonicotinoids

The biodegradation of neonicotinoids depends on the structure of the insecticide and the catabolic activity of the degrading microorganisms (Hussain *et al*., 2016). It is determined by the individual substituents of the insecticide (Tomizawa and Casida, 2005). The microbial degradation of neonicotinoids is the most efficient and environmentally friendly repair pathway (Hamada *et al*., 2019). An array of neonicotinoid-degrading microorganisms has been isolated and identified. Thirteen different pure bacterial strains were reported to degrade imidacloprid (Akoijam and Singh, 2015), where nine of them degraded acetamiprid, three of them thiacloprid, and two of them degraded thiamethoxam (Hussain *et al*., 2016).

2.3.6 Carbamates

Carbamates were introduced in the early 1950s and were made available in markets around 1970. Carbamates are a wide spectrum of biologically active pesticides used worldwide to control insects and nematodes. They are used against a wide range of insects such as leaf monors, cockroaches, ants, scale insects, mealy bugs, and white flies. The characteristics of carbamates are high polarity, soluble in water and thermally unstable. Carbamates consist of mainly three groups: -

- N-methyl carbamate ester of phenols
- N-methyl and N- dimethyl carbamate esters of heterocyclic phenols.
- Oxime derivatives of aldehydes.

They are classified into N-methyl carbamate and N-alkyl carbamate herbicides based on chemical structure and biological actions (Parks *et al*., 1987; Ozturk *et al.,* 2016). The first carbamate insecticide, Carbaryl was introduced in 1956. The pesticides come under the category of carbamates are: - carbofuran, carbaryl, aldicarb, propoxur, oxamyl, methiocarb, carbendazim, primicarb, carbosulfan, and fenoxycarb.

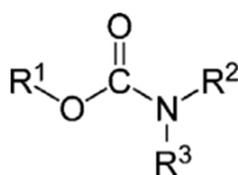


Figure 2.8 Chemical Structure of Carbamates

The mode of action of carbamates is like that of organophosphate insecticides that they are the inhibitors of acetylcholine esterase. They are the reversible inhibitors of acetylcholine, and which act as acetylcholine complexes and block the hydrolysis response of AChE in both synapses and neuromuscular junction that leads to disorders of the central nervous system (Vidair, 2004; Berman *et al*., 2017). They have very low vapour pressure and low evaporation at room temperature.

2.3.6.1 Toxicity of Carbamates

The carbamates are absorbed via oral or dermal route and the toxicity time is shorter than OPPs due to the reversibility of AChE. Anguiano *et al*., (2017) reported that carbamates are responsible for poisoning and the prevalent occurrence of an infectious disease in many developing countries. The clinical toxicity of carbamates depends upon the type of carbamate used, the dose, and its exposure level (Lamb *et al*., 2016). Due to their acute toxicity, they are poisionous to living organisms (Gupta, 1994) and highly toxic to plants and animals (Chin-Pampillo *et al*., 2015). They affect the soil microflora and soil productivity (Gupta *et al*., 2016). Carbamates are very stable in aquatic conditions, as a result, excessive pesticide application results in the major reduction of aquatic organisms. Carbamates are toxic to animals and human beings. Carbaryl, a carbamate insecticide act as a ligand for the hepatic aryl hydrocarbon receptor, a transcription factor involved in the mechanism of dioxin poisoning (Denison *et al*., 1998). They are associated with endocrine-disrupting activity (Goad *et al.,* 2004), reproductive disorders (Mnif *et al*., 2011; Jamal *et al*., 2016), exhibits cytotoxic and genotoxic effects on hamster ovarian cells (Soloneski *et al*., 2015), induce apoptosis and necrosis in human cells, natural killer cells (Li *et al*., 2012), dementia (Lin, 1996) and non-Hodgkin's lymphoma (Zheng *et al.,* 2001). Carbamate pesticides have detrimental effects on the immune system. It intervenes with metabolism, signal transduction pathways and cellular structures of the immune

system (Banks and Lein, 2012). The metabolism of carbamates caused the generation of Reactive Oxygen Species (ROS) (Lasram *et al*., 2014).

2.3.6.2 Degradation and Metabolites of Carbamates

Carbamate pesticides are transformed into several products through different process such as oxidation, biotransformation, hydrolysis, bio-augmentation, photolysis, and biodegradation (Cai *et al*., 2015). The major detoxification routes of carbamates are hydrolysis and oxidation. Hydrolysis is the primary phase of metabolic degradation of carbamates in soil.

The hydrolysis mechanism is different for both N-methyl carbamate and N-dimethyl derivatives (Chanika *et al*., 2011). The N-methyl carbamates metabolize to isocyanate metabolites and N-dimethyl derivatives to alcohol and acid (Rosman *et al*., 2009). Carbamates undergoes successive oxidation process like hydroxylation of the aromatic ring, N-dealkylation, oxidation of aliphatic side chains, O-dealkylation, N-methyl hydroxylation and sulfoxidation to sulfone (Chaudhry and Ali, 1988; Otieno *et al*., 2010). Generally, the metabolites of carbamates are less toxic than its parent compound, but in some cases, the metabolites are more toxic than the original compound. Bansal, (2005) found that the degradation of carbamate increased with soil organic matter, soil surface area, percentage of clay content, temperature, soil moisture, and soil pH. The carbamate pesticides were biodegraded and metabolized by diverse microbial consortia in the environment (Onunga *et al*., 2015; Satish *et al*., 2017). Esterases and amidases are the two major enzymes that hydrolyze carbamate pesticides. Hydrolysis by esterases is the most effective detoxification route.

2.4 Quinalphos – An organophosphate Insecticide

Quinalphos is a widely used pesticide that acts as a non-systemic broad-spectrum insecticide and acaricide. It is introduced in the year 1969 and Indian agriculture sectors use Quinalphos over certain crops such as cotton, groundnut, coffee, sugarcane, ornamental crops, and rice (Babu *et al.,* 1988; Jena *et al*., 1990). It has a high level of potency against a wide range of chewing, sucking, biting, and leaf-mining pests from Lepidoptera, Diptera, Coleoptera, and Hemiptera. In India, quinalphos come under the yellow-labeled (highly toxic) category.

Table 2.8 Physical and Chemical properties of Quinalphos

Chemical formula	$C_{12}H_{15}N_2O_3PS$
Molecular Mass	298.3
Pesticide type	Insecticide, Acaricide
Substance group	Organophosphate
Substance origin	Synthetic
Mode of action	Direct contact and stomach action, Non-systemic Acetylcholinesterase (AChE) inhibitor
IUPAC name	O, O-diethyl O-quinoxalin -2-ylphosphothioate
Physical state	Colorless crystals
Parent compound	Quinoxaline-2-ol
Melting point	31.5°C
Density	1.235g/ml
Boiling point	Unknown
Solubility in water	17.8mg/L
Solubility in Organic solvents	Soluble in Hexane

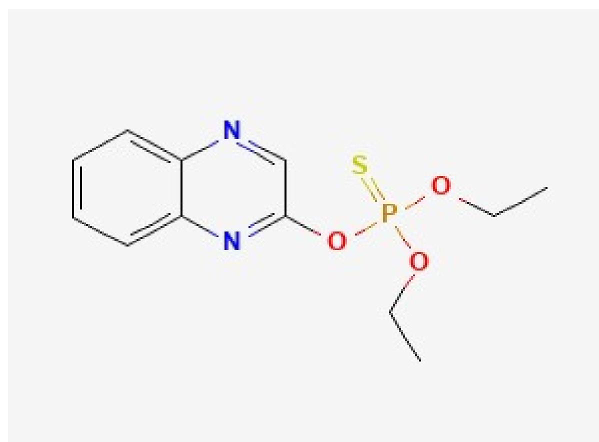


Figure 2.9 Chemical structure of Quinalphos

2.4.1 Toxicity of Quinalphos

Quinalphos is ranked in class II moderately toxic substance by World Health Organisation (WHO) and Environmental Protection Agency (EPA). It shows Class II toxicity levels (Srivastava *et al*., 2000). Mancini *et al*., (2005) show that more than

eighty percent (83.6%) of mild to severe Quinalphos toxicity is reported in India whereas, one-tenth shows neurotoxic symptoms. Quinalphos shows acute oral toxicity LD50 of 71mg/kg in rats. (Dwivedi *et al*., 1998) reported the toxicity of quinalphos to rats and other animals. The toxicity of quinalphos depends on several factors, when temperature increases the toxicity levels also increase (Satpute and Barkhade, 2012). High levels of quinalphos are hazardous to (silver barb), *Barbonymus gonionotus* (Sadiqul *et al*., 2016). Quinalphos inhibits the activity of AChE in the foetal brain and placenta of pregnant rats when supplemented at doses of 1.5mg/kg body weight (Srivastava *et al.,*1992). The experimental studies of (Ray *et al*., 1992) show that quinalphos affects the testicular steroidogenic enzymes which result in the degeneration of germ cells and a decrease in sperm count in rats. There are several metabolites for quinalphos and the desertification of quinalphos produces quinoxalin-2-ol. In most cases, this metabolite is excreted through urine, but it is also retained in the bile duct. Debnath and Mandal, (2000) the study suggests that quinalphos is an environment xenoestrogenic insecticide that causes mammalian toxicity. Quinalphos affects the stomach and respiratory systems of non-target organisms (Yashwantha *et al*., 2016). The excessive use of quinalphos affects the non-target organisms (Vig *et al*., 2006; Chebbi, 2009). It adversely alters the esterase activity in the blood and brain of chickens (Vairamuthu *et al*., 2003) and decreases fertility efficiency in adult male rats (Sarkar *et al.,* 2000). The soil microbial population is inhibited by the unlimited use of pesticides. Quinalphos obstructs the fungal population in the soil samples (Pandey and Dileep, 2004) and it is toxic to aquatic organisms, especially crustaceans and fishes.

2.4.2 Major metabolites of Quinalphos

The metabolite of Quinalphos is studied for a long time ago. The two major metabolites are 2-hydroxyquinoxalin ($C_8H_6N_2O$) and Phosphoric acid (H_3PO_4). The degradation studies done so far by research resulted in several metabolites of quinalphos in different matrices like water, soil, and various substrates. Pawar and Mali, (2016) studied the degradation of quinalphos in soil samples and reported about four metabolites, Butanoic acid, 3-methyl, butanedioic acid, thiazol,4, -dihydro-2-methylamino, phosphoric acid and trimethyl ester. Talwar *et al*., (2014) reported the metabolites 2-hydroxyquinone and diethylphosphate. 2-hydroxyquinoxaline (2-HQ) is the main hydrolytic metabolite in water, soil, and crops (Menon and Gopal, 2003). In

Quinalphos the metabolite is formed by the hydrolysis of the ester bond to the aromatic moiety to dimethyl phosphorothioate in quinalphos. 2-HQ is formed by pH-independent hydrolysis, which is mediated by microbes (Menon and Gopal, 2003), by photodegradation (Dureja *et al*., 1988), and by photolytic degradation (Goncalves *et al*., 2006). (Gupta *et al*., 2011) reported that 2-HQ is more toxic compound than the parent compound. The photolytic degradation of quinalphos resulted in the formation of hydroxylated quinalphos, quinalphos-oxon and dealkylation of quinalphos moiety (Kaur and Sud, 2012). In most of the studies, the microbial degradation of quinalphos results in the formation of 2-HQ (Talwar *et al*., 2014; Gangireddygari *et al*., 2020). The enzymatic hydrolysis of quinalphos within the organisms results in the formation of metabolites like, Quinalphos Oxon, O-ethyl-O-quinoxaline, phosphoric acid, 2-HQ, and ethyl phosphoric acid (Gupta *et al*., 2012). Hydrolysis of quinalphos follows two pathways and forms 2-HQ and diethylquinalphos (Pusino *et al*., 1988). The amount of metabolite desethylquinalphos formed by the loss of ethyl group is high in the beginning of every experimental studies. The predominant metabolite in soil is O, O-diethyl-O-methylphosphorothioate (Goncalves *et al*., 2006).

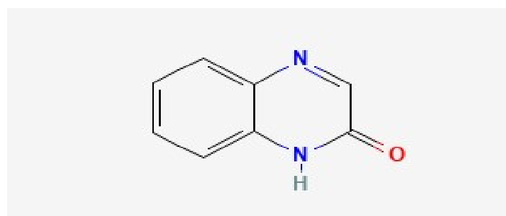


Figure 2.10 Chemical structure of 2-hydroxyquinoxaline(2-HQ)

2.4.4 Degradation Process of Quinalphos

The quinalphos is degraded by biotic and abiotic methods. Photodegradation, photolytic degradation, hydrolysis and volatilization are the major degradation process of quinalphos. The enzymes mediate the degradation and detoxification process of quinalphos naturally. Phosphotriesterases (PTEs) and carboxylesterases (CBEs) are the organophosphate hydrolase enzymes (OPH) responsible for this degradation process (Sogorb and Vilanova, 2002). Photodegradation is an abiotic process by which pesticides are dissipated to less or more complex molecules by the excitation of the pesticide in the presence of light. Quinalphos is photodegraded in distilled water, tap water, rainwater and in aqueous acetone by the exposure to natural sunlight. The half-life of quinalphos is one month in distilled water, twenty to twenty-

three days in tap water and rainwater and less than five days in 2% aqueous acetone (Dureja *et al.*, 1988). In water, it shows first order kinetics with half-life 11.6 to 19.0 hours. Goncalves *et al*., (2006) studied the degradation kinetics of quinalphos in soil matrices, it shows two step pseudo first order photoreaction. The products formed were 2-HQ, s-triethylthiophosphate and triethylphosphorothiate and the half-life ranges from 16.9 to 47.5 hours. About 89% of quinalphos is degraded photolytically in the presence of TiO_2 at a dose of 1.5g/L. Its photochemically transferred to different metabolites of quinalphos and has maximum degradation at the pH 8 (Kaur and Sud, 2012).

Hydrolysis is the most common chemical reaction for the abiotic degradation of pesticides. It consists of the cleavage of weak phosphate ester bonds of the quinalphos. Quinalphos follows two pathways in hydrolysis reaction (Menon and Gopal,2003) one in the presence of nucleophilic attack at the aliphatic carbon in the presence of Na, K, and Ca clays, and another one in the formation of diethylquinalphos. Pusino *et al*., (1988) studied the homoionic hydrolysis pathway of quinalphos in the presence of Na, K, Ca, Cu, Cl, Al, and Fe-bentonites. Quinalphos is hydrolyzed into 2-HQ under controlled conditions like pH 11.8 – 13.6, the temperature at 25°C to 45°C in an aqueous solution. Volatilization is an important process in the dissipation of organophosphate compounds. The volatilization process is affected by several factors like vapor pressure, solubility, adsorption, and persistence of the compound (Racke *et al*., 1997) and volatilization of quinalphos is moderate, with VP of $3.6x10^{-6}$, when compared to other organophosphate compounds. The degradation studies of quinalphos are done in-vivo and in-vitro in rats. The degradation was done in both the gastric phase and intestinal phase of rats and resulted in the formation of its metabolites. The degradation followed the first-order kinetics, the rate of degradation is faster in the gastric phase because of its acidic pH and the presence of enzymes pepsin and pancreatic enzymes (Gupta *et al*., 2012).

2.4.4.1 Biodegradation of Quinalphos

The pesticide quinalphos is degraded by the biotic components mainly by microbes in various matrices. The bacterial species Ochrobactrum sp. is isolated from soil samples of Karnataka fields which show a degradation potential of 84.6% at pH 7 and at 27°C. It resulted in the formation of metabolites 2-HQ and diethylphosphate (Talwar *et al*.,

2014). Dhanjal *et al., (*2014) reported quinalphos degrading *Bacillus sp*. and *Pseudomonas sp.* from soil samples of Punjab. Nearly fourteen strains of *Pseudomonas* species from vineyard soils of Maharasthra show the quinalphos degradation potency of 90.4% in the presence of co-substrate glucose (Pawar and Mali, 2014). The bacterial species belonging to *Pseudomonas, Serratia*, and *Pseudomonas aeruginosa*, show degradation potency at the rate of 86%, 82%, and 94% respectively (Nair *et al.*, 2015). Gangireddygari *et al.*, (2017) studied the degradation potency of *Bacillus subtillus* and *Bacillus thuringiensis* at conditions like OD-1, pH-6.5-7.5, and temperature 35-37°C, from vineyard soil samples. A rhizosphere bacterium *Kosakinia oryzae* from wetland paddy fields shows the degrading ability of profenofos and quinalphos (Dash and Osborne, 2020). *Acinetobacter sp*.7-13 is isolated from grape vineyards contaminated with quinalphos and it shows a degradation potency of 87.82% within 7 days at the temperature 35°C, pH 7, and pesticide concentration 15000µg/L and degraded quinalphos to its metabolites butanoic acid, butanedioic acid, thiazol,4,5-dihydro-2-methylamino and phosphoric acid and trimethyl ester (Pawar and Mali, 2016).

2.4.4 Quinalphos Residue Analysis Techniques and Sites

The insecticide quinalphos contaminated areas are identified and studied by researchers in India. All the matrices like water, soil, vegetables, and plants are polluted with the residues of quinalphos. Quinalphos is detected in soil samples from paddy and wheat growing areas at Haryana by the techniques GC-ECD and GC-NPD (Kumari *et al*., 2008) and cardamom soils from Idukki by GC-MS (Uma *et al*., 2014). It is presented in tea leaves in Tamil Nadu and detected by GC-MS (Kottiappan *et al*., 2013). The residue of quinalphos is analysed through instruments like GC-ECD, GC-NPD, GC-MS in vegetables at the places like Haryana, Delhi, Jaipur, Himachal Pradesh (Mukherjee, 2003; Mandal and Singh, 2010) and in honey (Choudary and Sharma, 2008).

2.5 Glyphosate – A Herbicide

Glyphosate is also known as glyphosate acid, sulphosate, 2-[(phosphonomethyl) amino] acetic acid, and CP67573 was introduced in the year 1971 and it is approved by EU 1107/2009 status. It is a non-selective and common herbicide. It controls annual and perennial weeds. Organophosphate herbicide and phosphoglycine

herbicide are the substance groups of Glyphosate. Glyphosate was discovered in 1970 and accepted as an herbicide in 1974. It was synthesized by Henri Martin Swiss pharmaceutical company (Gilag). Its herbicidal property was described by John. E Franz of Monsanto company (Gill *et al*., 2017). Glyphosate is always combined with several surfactants & manures to increase its ability to destroy unwanted weeds. Various survey reports show that the annual consumption of glyphosate in the last two decades has increased to 240 million pounds. It is the most used herbicide in the USA and in Germany and Denmark (Steinmann *et al*., 2012) and Argentina (Nedelkoska and Low, 2004). Glyphosate is a polyprotic molecule with three polar functional groups: amino group, phosphate, and carboxyl group (Gill *et al*., 2017).

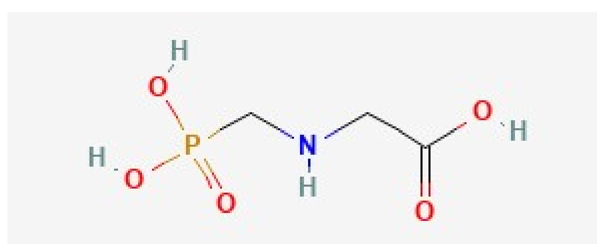


Figure 2.11 Chemical structure of Glyphosate

Table 2.9 Properties of Glyphosate

Chemical formula	$C_3H_8NO_5P$
Molecular Mass	169.1
Pesticide type	herbicide
Substance groups	Organophosphate herbicide, phosphonoglycine herbicide
Minimum active purity	>=950g/kg
Substance origin	Synthetic
Mode of action	Broad-spectrum, systemic, contact action translocated and non-residual, inhibition of EPSP synthase
IUPAC name	N-(phosphonomethyl)glycine
Physical state	White crystals
Melting point	189.5^{o}C
Boiling point	Decomposes before boiling
Density	1.71g/ml
Solubility in water at 20°C	100000mg/L
Solubility in organic solvents at 20°C(mg/l)	Methanol>Acetone=xylene=Ethyl acetate

2.5.1 Mode of Action

Glyphosate kills unwanted weeds by inhibiting the biosynthesis of essential aromatic aminoacids required for its growth. The Shikimate pathway is a metabolic pathway present in plants for the biosynthesis of aromatic amino acids (Gill *et al*., 2017). Glyphosate retards the activity of the main enzyme, 5-enolpyruvylshilimate-3-phosphate synthase of the shikimate pathway by acting as an antagonistic analog of phosphoenol pyruvate. Low enzyme activity leads to the deficiency of aromatic amino acids and hence destroys the plant (Tu *et al*., 2001). This enzyme plays a significant role in the biogenesis of chorismite – an intermediate in the synthesis of aromatic amino acids (Williams *et al*., 2000). The uptake of glyphosate by plant surfaces such as plant cuticle and leaf vary from species to species (Kirkwood *et al*., 2000).

2.5.2 Toxicity of Glyphosate

The excess use of glyphosate possesses serious threat to lower invertebrates and higher vertebrates. Recent data shown that glyphosate possess genotoxicity (Bolognesi *et al*., 2009) and cutaneous toxicity (Nagami *et al*., 2005). The International Agency for Research on Cancer (IARC) classified glyphosate in Group 2A (probable human carcinogen) in March 2015. Continuous use of glyphosate causes deleterious effects on the soil system by inhibiting the growth of microorganisms (Carlisle and Trevors, 1988). It retains in topsoil layers due to its strong sorption on clay, iron, and aluminium oxide particles (Vereecken, 2005; Borggaard and Gimsing, 2008; Rampazzo *et al*., 2013; Okada *et al*., 2016; Sidoli *et al*., 2016). Microbial population is significantly decreased when Glyphosate is added (Santos and Flores, 1995; Kryzsko-Lupicka and Orlik, 1997), but the growth stimulation of microbes is high when applied in high concentrations (Dick and Quinn, 1995; Liu *et al*., 1991). The half-life of Glyphosate varies from 2 to 197 days in soil depends on soil type, climate conditions, and the level of microbial activity (Sorensen *et al*., 2006).

Oliveira *et al*., (2007) studied glyphosate toxicity in both the spatial and temporal distribution of *Nitella microcarpa* in the ecosystem. It is toxic to several common algal species (Tsui and Chu, 2003).

Glyphosate is toxic to several lower invertebrates like *Nemathelminthus*, toxic to annelids like earthworms (Verrel and Van Buskirk, 2004; Casabe *et al*., 2007; Yasmin and Disoyza, 2007; Piola *et al*., 2013; Santadino *et al*., 2014;; Zaller *et al.,* 2014;

Gaupp-Berghansen *et al*., 2015; Salvio *et al*., 2016; Alcantara *et al*., 2016; Griffiths and Datla, 2019). Glyphosate has many ruminous effects on Arthropods, one of the biggest groups of invertebrates. It poses severe toxic impacts on crustaceans like *Daphnia* (Hartman and Martin, 1984; Alberdi *et al*.,1996; Szekacs *et al*., 2004; Dominguez Cortinas *et al*., 2008; Cuhra *et al*., 2013). Dutra *et al*., (2011) observed the toxic effects of glyphosate on freshwater amphipoda *Hyalella castrol*. Glyphosate decreased the lipid & protein level in muscles of crayfish (Avigliano *et al*., 2014).

Along with unwanted weeds, glyphosate also suppresses the growth of all plants which are not genetically resistant to them. The overuse of glyphosate severely affected the non–target organisms in the soil environment (Friends of Earth Europe, 2013). Earlier, glyphosate was regarded as non-carcinogenic in nature (Duke and Powles, 2008). WHO and FAO-reported that glyphosate causes non-Hodgkin's lymphoma in some case studies. At higher doses, Glyphosate could imitate cancer in rats (Gill *et al.,* 2017). Glyphosate exhibits toxicity in multicellular organisms found in both soil and water. Glyphosate is toxic to several algal species such as it decreased the chlorophyll content in two algal species of *Scenedemus* (Saenz *et al*., 1997) and in macro algae and seagrass species of marine aquatic systems (Kittle *et al*., 2018).

When used in the recommended dose, glyphosate has no effects on non–target organisms expect some species of fungi (Franz *et al*., 1997). It is toxic to unicellular organisms. Glyphosate decreased the chlorophyll content and respiration rates in *Euglena gracilis* (Richardson *et al*., 1979). At concentrations above 10 ppm to 5000 ppm glyphosate affected the growth rate of ectomycorrhizal fungal species (Chakravarty and Sidhu, 1987). Glyphosate had a negative impact on the growth of rhizospheric microbial communities such as *Pseudomonads* (Zobiole *et al*., 2011) and *Acidobacteria* (Newman *et al.,*2016). Intake of glyphosate reduced the population count of beneficial poultry bacteria (Shehata *et al*., 2013). Leaching of glyphosate from soil to waterbodies has also affected the organisms present in the periphyton communities (Goldsborough and Brown, 1988) and diatoms in lotic systems (O'Sullivan *et al.,*1981).

Glyphosate has adverse effects on frogs and toads (Mann and Bidwell, 1999; Howe *et al.,*2004; Lajmanovich *et al*.,2011; Lajmanovich *et al*., 2015; Bach *et al*., 2016; Dornelles and Oliveira, 2016; Perez- Iglesias *et al*., 2016; Soloneski *et al*., 2016).

Different Research groups examined the devastating effects of glyphosate on reptiles, such as in crocodiles (Poletta *et al.,* 2009; Siroski *et al.,*2016), in lizards (Carpenter *et al.*, 2016; Schaumburg *et al.*, 2016). Linz *et al.*, (1996) illustrated that glyphosate reduced the wetlands vegetation which gradually affected the bird population. Santillo *et al.*, (1989) exemplified that the use glyphosate affected the versality of the birds and reduced their breeding population. Oliveira *et al.,* (2007) analysed the effect of glyphosate on the reproduction of bird species. Tizhe *et al.*, (2014) observed severe histopathological changes in rats when exposed to higher concentration of glyphosate and he also assessed the sub chronic toxic effects of glyphosate on Wistar Rats. Dallegrave *et al.*, (2007) evaluated the effects of glyphosate on the reproductive behavior of Wistar rats. Romano *et al.*, (2010) evaluated that glyphosate reduced the testosterone production in male rats. Glyphosate caused detrimental effects on the cardiovascular system of the swine (Lee *et al.*, 2009).

The toxicological impact of glyphosate on honeybees was examined by different researchers all over the world. Glyphosate terminates the potential gut bacteria in honeybee (Burlew, 2010). Glyphosate decreased the elemental learning & damaged the non-elemental associative learning in honeybees (Herbert *et al.,* 2014). Balbuena *et al.*, (2015) revealed that glyphosate impaired the intellective capability of honeybees. Gregorc, (2012) detected that glyphosate triggered cell death of honeybee larvae. Glyphosate is toxic to several aquatic and terrestrial snails (Tate *et al.*, 1997; Druart *et al.*, 2011). Glyphosate expressed injurious effects on sea urchins (Marc *et al.*, 2005). Researchers evaluated the toxic impact of glyphosate on different types of fish such as Rainbow trout (Folmar *et al.*, 1979), *Cyprinus carpio* (Neskovic *et al.*, 1996), Piava fish (Salbego *et al.*, 2010) *Cyprinus carpio* (Cattaneo *et al.,* 2011), *Cnesterodom* (Menendez-Helman *et al.,* 2012), *Jenynsia multidentata* (Hued *et al.*, 2012), fresh water fish surubim (De souza filho *et al.*, 2013), brown trout, and on silver catfish (Murussi *et al.,* 2016)

2.5.3 Metabolites of Glyphosate

The key metabolite of glyphosate is aminomethylphosphonic acid. Aminomethyphosphonic acid is the main degradation product of glyphosate (Figure 2.12).

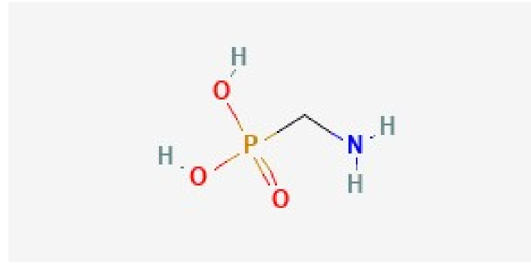


Figure 2.12 Chemical structure of aminomethylphosphonic acid

2.5.4 Degradation of Glyphosate

Degradation and absorption of glyphosate mainly depend on soil properties, including their phosphorous states (Gimsing *et al*., 2004; Laitinen *et al*., 2009). It is more resistant to photolysis and chemical decomposition (Mallat and Barcelo, 1998; Singh and Walker, 2006). Soil microbes degrade glyphosate in soil (Nomura and Hilton, 1977; Rueppel *et al*., 1977) Glyphosate herbicide introduced in 1980's is degraded by *Pseudomonas putida* and *Burkholderia* (Kuklinsky *et al*., 2005). *Pseudomonas sp.* strain LBr degraded high levels of glyphosate by converting it to (AMPA) aminomethyl phosphonate (Jacob *et.al.,* 1988) *Pseudomonas strain* PG2982 degrade glyphosate resulting in the release of sarcosine and phosphate group (Jacob *et al*., 1985; Kishore and Gary, 1987), and *Arthrobacter sp.* Strain GLP-1 (Pipke *et al.,*1987) degrade glyphosate by the production of glycine. *Flavobacterium s*p strain GD-1 and mixed bacterial culture from soil (Nomura and Hilton, 1977; Rueppel *et al*., 1977; Balthazor, 1986) degrade glyphosate by producing AMPA. Many microorganisms are reported with the potential to degrade glyphosate are given in Table Schulz *et al*., (1985) isolated five glyphosate degrading isolates of *Pseudomonas species P. maltophilia, P. putida*, and *P. aeruginosa*..

Due to the stable C-P bond glyphosate is resistant to chemical hydrolysis, thermal decomposition, and photolysis (Kononova and Nesmeyanova, 2002). Several research studies demonstrated that glyphosate degradation seems to occur in fungi and plants (Duke, 2011; Rojano-Delgado *et al*., 2012; Vemanna *et al*., 2017; Pan *et al*., 2019). Glyphosate reaches the soil in three ways. One by direct application of glyphosate to the soil surface, second released by plant roots, and finally emitted from the decomposed plant. It undergoes various chemical and physical changes in the soil. When compared to other pesticides the sorption of glyphosate to the soil is large because it is a polyprotic molecule. Al-Rajab *et al*., (2008) found that only 5-24% of

glyphosate is initially sorbed and the rest remains in the soil. Glyphosate forms chelates & complexes with metal ions in the soil (Vereecken, 2005) and it is degraded by microorganisms present in the soil. The biological degradation of glyphosate takes place by different pathways, by the enzymatic reactions of enzymes such as glyphosate oxidoreductase, C-P lyase and glycine oxidase. The degraded reaction products are glyoxylate, amino methyl phosphonic acid, methylamine, inorganic phosphate, sarcosine formaldehyde and glycine. Glyoxylate, methylamine and glycine were consumed by microorganisms (Dick and Quinns, 1995). Microbial degradation of glyphosate is quite slow because it is strongly adsorbed by the soil and the average half-life of glyphosate is two months. Tu *et al*., (2001) reported that degradation of glyphosate was affected by the type of microbial community present in the soil and was easily degraded by the enzymes released by the microbes. Glyphosate - degrading metabolic processes was observed in several bacterial species (Jacob *et al*., 1985). Moore *et al*., (1983) studied the breakdown of glyphosate into phosphorus by *Pseudomonas* PG2982 strain. Other microorganisms like *Rhizobium meliloti*, *ArthrobacterGLP-1* strain, *Agrobacterium radiobacter* and *Rhizobium* strains also degrade glyphosate (Pipke *et al*., 1987; McAuliffe *et al.,*1990; Lin *et al*., 1991; Dick and Quinn, 1995). Pipke and Amrhein, (1988) isolated a bacterial strain *Arthobacter* GLP-1/Nit -1 that utilizes glyphosate as a nitrogen source. *Streptomyces* utilizes glyphosate as both phosphorus and nitrogen (Obojska *et al*., 1999). Lane *et al*., (2012) explained that the presence of phosphorus in glyphosate is responsible for its microbial degradation.

2.6 Ethion – an Organophosphorus insecticide

Ethion [o,o,o,o-tetraethyl S-S methylene – bis-phosphorodithioate] is an organophosphorous (compound with a thiophosphoryl (P=S) functional group) non-systemic insecticide, ovicide and acaricide with knockdown and long-lasting residual activity against pests of tea, cotton, vegetables etc. It is introduced in the year 1956 by Food Machinery and Chemical Company (FMC). As per WHO, ethion is moderately hazardous.In 1989, 1 million pounds were used in the US, and in 1992, 868,218 pounds of ethion were used for farming. As per the reports of 2017 to 2018 the usage of ethion in the country is 20.73 metric tonnes. In tropic areas, ethion is extensively used for tea cultivation. It affects the target animals by inhibiting the acetylcholinesterase (AChE) enzyme (Karalliedde and Senanayake, 1989; Leili *et*

al.,2016). The maximum residue level (MRLs) for ethion is 0.5mg/kg (Maddah and Hassanzadeh, 2017).

Table 2.10 Physical and Chemical properties of ethion

Chemical formula	$C_9H_22O_4P_2S_4$
Molecular mass	384.48
Pesticide type	Insecticide, acaricide, metabolite
Substance groups	Organophosphate insecticide and acaricide, organothiophosphate insecticide and acaricide.
Substance origin	Synthetic
Mode of action	Non-systemic with a predominate contact action, AChE inhibitor
IUPAC name	O,O,O’,O-tetraethylS,S’-methylene bis(phosphorodithiote)
Physical state	Amber liquid
Melting point	-12^oC
Boiling point	165^oC
Density	1.22g/ml
Solubility in water (at 20^oC)	2mg/L
Solubility in organic solvents (at 20^oC)	Miscible in acetone, ethanol, xylene, methanol.

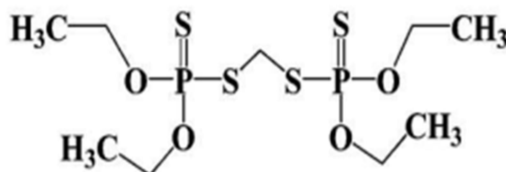


Figure 2.13 Chemical structure of ethion

2.6.1 Persistence and Toxicity

It is classified as Toxicity Class II moderately toxic. Ethion is moderately or highly persistent in soil (Foster *et al*.,2004). Ethion degraded faster with the increase in the pH of the medium (Melinkov, 1971). It is hydrophobic in nature, as a result it gets easily adsorbed to soil, sediments and organic matter. Due to high adsorption the runoff of ethion to natural water systems was reduced (Sharom *et al*.,1980; Xia and Ma, 2006). (Barzi *et al*., 1996) reported that the ethion concentration up to 45g/kg in environment which is unacceptable than the permissible limit. Ethion persist longer period in acidic and laterite soils and it shows half-life of 22 days (Melinkov, 1971). In India residues of ethion is detected in tea garden soils of West Bengal (Bishnu *et al*., 2009). It is used in the tea cultivation at tropical areas and highly persistent in soil.

Ethion is moderately toxic to soil microflora, and it inhibits the LDH activity in different microorganisms like *Rhodococcus* Sp. AK 1, *Rhizobium* and *E. coli* HB 101

(Kalam and Mukherjee, 2000). Ethion affects the germination of *Passiflora edula* (Silva *et al.,*1999). It exhibits a mammalian toxicity D50 of 50mg/kg of body fat. Ethion has moderate effect on Total Microbial population (TMC) and inhibited the activity of GDH enzyme in Rhizobium, *B.subtilis, Rhodococcus* and *E.coli.*

Ethion induces toxicity in economically important silkworms by altering the carbohydrate metabolism. It decreases the activity of pyruvate levels and lactate dehydrogenase enzymes. The mobilization of pyruvate into the Kreb's cycle is reduced by the exposure of ethion (Nath, 2000). The lethal and sublethal doses of ethion resulted in the depletion of fat body glycogen reserves in silkworm, Bonbys mori. Ethion increased the glycogenolysis at tissue level. The exposure of ethion caused the failure of haemostatic mechanism in silkworms and it also produced hyper-hypo trehalosemia in hemolymph and hyper-hypoglycemia in fat body (Nath, 2002).

2.6.2 Degradation of Ethion

Dierberg and Pfeuffer, (1983) demonstrated the hydrolysis and biodegradation of ethion in a variety of environments. In buffered distilled water the half -life of ethion is 20.8 weeks. Ethion degrades faster when the pH of the medium is high (Melinkov,1971). Ethion degrades faster in the field than the laboratory conditions (Digrak *et al*.,1995). Ethion degrades faster in hilly soils and at high temperatures because of the increased microbial activity in the soils (Bishnu *et al*., 2012). Several studies are done on the degradation of ethion. H_2S is produced by ethion by bacteria in lagoonal sediments (Sherman *et al*.,1974). Ethion is degraded by phytoremediation by water hyacinth (Xia and Ma, 2006). *Pseudomonas* and *Azospirillium* species isolated in Australian landfills degrade ethion (Foster *et al*., 2004). Abd-di Ghany and Masmali, (2016) reported the degradation of ethion fungus. Kan *et al*., (2021) reported the oxidation remediation of ethion. The abiotic hydrolytic degradation metabolites of ethion are; ethion monoxon, ethion dioxon, O, O-diethylthiophosphate and thio-formaldehyde (Ivey and Mann, 1975; Dierberg and Pfeuffer, 1983;).

2.7 Deltamethrin- the Pyrethroid insecticide

Deltamethrin is one of the most popular and widely used pyrethroid insecticides since 1978 (Katsuda, 1999) and that is used against a broad spectrum of insect pests of economically important crops and against household insect pests (Karpouzas and

Singh, 2006). It is synthesized in 1974. Deltamethrin disrupts the cellular calcium and sodium channel, and it is immobile and soluble in the environment and water respectively (Muraleedharan *et al.,*1995). Deltamethrin has been widely used for more than 30 years on various crops and vegetables for pests such as mites, ants, weevils, and beetles (Cycon *et al*., 2014). In India and other developing countries, deltamethrin is also used to control human disease vectors, such as mosquito species that carry Zika and the dengue virus (WHO, 1990; Yadav *et al*.,2001). The formulations of deltamethrin are emulsifiable concentrate, wettable powders, flowable formulations and granules.

Table 2.11 Physical and chemical properties of Deltamethrin (Zhang *et al*.,1984)

Chemical formula	$C_{22}H_{19}Br_2NO_3$
Molecular Mass	505.2
Pesticide type	Insecticide, Metabolite, veterinary substance
Substance groups	Pyrethroid insecticide, pyrethroid ester insecticide
Minimum active purity	985g/kg
Substance origin	Synthetic
Mode of action	Non-systemic with contact and stomach action, sodium channel modulator.
IUPAC name	(s)-α-cyano-3-phenoxybenzyl(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate
Physical state	Colourless crystals
Parent compound	Tralomethrin
Melting point	101°C
Density	0.55
Boiling point	Decomposes before boiling
Solubility in water(20°C)	0.0002mg/L
Solubility in organic solvents	Acetone>Xylene>Methanol>n-heptane

2.7.1 Biodegradation of Deltamethrin

Microbial degradation is the significant breakdown route of deltamethrin. Deltamethrin is degraded by both hydrolysis and microbial activity in the soil, but

slower under anaerobic conditions (Cycon *et al*., 2014). Various study reports have shown that many soil bacteria could metabolize synthetic pyrethroids by their esterase enzymes and or by using them as carbon and energy sources (Cao *et al*., 2006; Singh and Walker,2006; Yang *et al*., 2009; Lakshmi *et al*., 2008, Chen *et al*.,2011 2012; Fenner *et al*.,2013). The studies of Smith and Glenn, (1986), Zhang *et al*., (1984) and Grant and Betts, (2004) revealed that deltamethrin degradation is slower under anaerobic conditions or sterile condition.

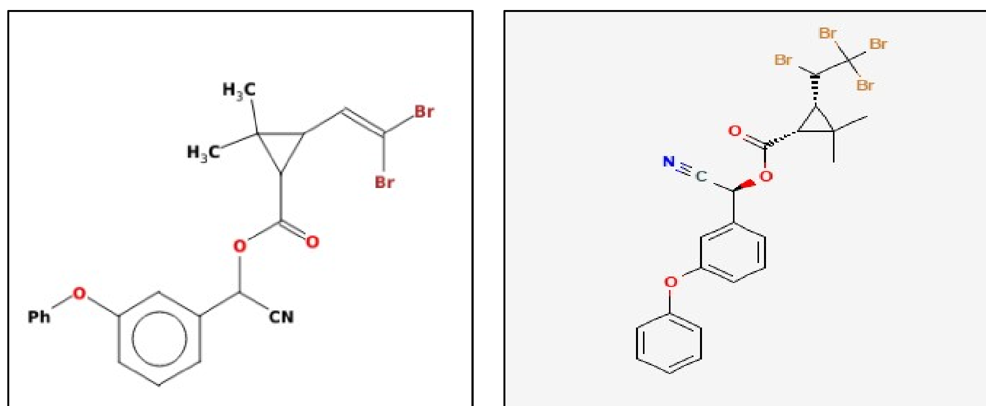


Figure 2.14 Chemical structure of Deltamethrin(a) and Parent compound Tralomethrin(b)(ChemPub)

This indicates the important role of microorganism in this process. Soil microorganisms were more responsible for the rapid rate of degradation in natural soil (Kaufman, 1981; Lee and Ward, 1985). *Streptomyces rimosus* strain degrades deltamethrin in agricultural soil (Khajezadeh *et al*.,2020). Kumral *et al*.,(2020) studied the deltamethrin and chlorpyrifos degradation potential of *Lactobacillus plantarum* (LB-1 and LB-2). Significant growth is found in LB-1 and it also exhibited enhanced hydrolysis activity. Degradation of chlorpyrifos and deltamethrin by LB-1 and LB-2 reached the values of 96 and 90% to 24 and 53% after three days. The degradation of deltamethrin is enhanced by the soil bioaugmentation with two different strains of *Serratia marcescenes.* (Cycon *et al*., 2013). Soil microorganisms play a major role in the degradation of deltamethrin (Zhang *et al*.,1984; Grant and Betts, 2004). The natural microbial community potentially biodegrades deltamethrin and 3-PBA and an increase in the population of *Nocardioides* sp and *Sphingomonas* sp is observed (Braganca *et al*., 2019).

There are several deltamethrin and pyrethroid degrading bacteria like *micrococcus*(Tallur *et al*.,2008) *Pseudomonas* (Farre *et al*., 2002; Jilani and Khan, 2006) *Sphingobium* (Guo *et al*., 2009) *Ochrobactrum* (Chen *et al.,* 2011 a) *Streptomyces* (Chen *et al*.,2011 b, 2012 a) *Stenotrophomonas* (Chen *et al*; 2011 c) and *Bacillus* (Chen *et al*.,2015), *Bacillus cereus* and *Pseudomonas fluorescens* species and bacteria from the *Achromobacter* genus (Maloney *et al*., 1988*), Lysinibacillus* sp-ZJ6 (Hao *et al.,* 2018), *Paracoccus* (Ning *et al.,* 2020),*Streptomyces rinosus* (Khajezadeh *et al*., 2020) *Bacillus cereus* (Zhang *et al*., 2016) *Bacillus subtilis* (Bhuvaneshwari *et al*., 2018) *Streptomyces diastaticus* (Janarthanan *et al*., 2018) R*hodococcus erythropolis* (Kumar and Syed, 2018) *Sphingomonas pancomobili*s species and the Moraxella genus among planktonic bacteria and *Burkholderia cepacian* and *Bacillus mycoides* species among benthic bacteria (Bhanu *et al*., 2011) and halotolerant *Enterobacter ludwiggi*. Deltamethrin undergoes microbial degradation in one to two weeks (Kidd, James 1991) and it is also characterised by the highest rate of degradation with a decomposition time up to 21 days (Bhanu *et al*.,2011). In degradation tests with deltamethrin as the only source of carbon and energy on bacterial strains from soils, the initial concentration was reduced by 35.77% within a week and 59.7 – 72.5% within two weeks. Kalwasinska *et al*., (2011) evaluated the deltamethrin biodegradation by mixed cultures of 25 neustonic and epiphytic bacteria isolated from the epidermis of the common reed.

2.7.2 Metabolites and degradation pathways of Deltamethrin

Deltamethrin is degraded into different metabolites by various modes of degradation. The major metabolites of deltamethrin are Br_2CA1 trans-hydroxymethyl -Br_2CA, and 3(-4-hydroxyphenoxy) benzoic acid (WHO 1990). Deltamethrin is first degraded to hydroxy-3-phenoxy-benzene acetonitrile and 3-phenoxybenzaldehyde with soil bacteria using carboxyl ester and then 3-phenoxybenzaldehyde is oxidized to 2-hydroxy-4-methoxy-benzophene (Chen *et al*.,2011 b). 3-phenoxybenzaldehyde was reported as an antimicrobial agent and it prevents the proliferation of bacteria (Shivlata and Satyanarayana, 2017). The bacterial degradation of 3-phenoxybenzaldehyde was studied by (Cycon *et al*., 2013) in soils in the presence of *Serratia marcescens.* It has higher mobility than parent compounds and causes widespread contamination (Chen *et al*., 2012 b). Meeker *et al.,* (2009) reported that 3-phenoxybenzaldehyde is classified as an endocrine-disrupting chemical. This toxic

metabolite is found to be degraded by bacteria *Streptomyces* (Chen *et al*.,2011 b) and fungi *Cladosporium* (Chen *et al*.,2011b). A total of six metabolic compounds of deltamethrin were reported by (Wu et al.,*2021*), as a result of bacterial degradation. They are 3-(2',2'-dibromovinyl)-2, 2-dimethylcyclopropane carboxylic acid, 3-phenoxybenzaldehyde, 3-phenoxybenzoic acid, 1,2-benzenedicarboxylic acid, phenol, and muconic acid.

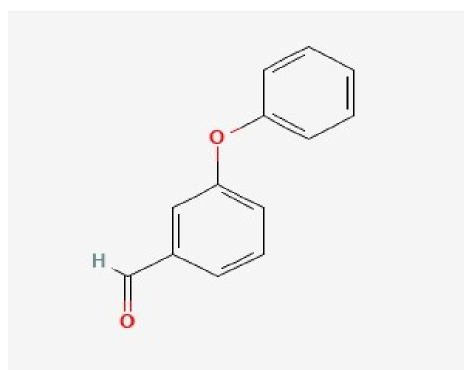


Figure 2.15: 3-phenoxybenzaldehyde

In animals and humans, deltamethrin metabolizes in diverse ways. The tissue esterase, distributed in the gut wall and liver of rats rapidly metabolizes the deltamethrin after oral administration (Myer, 1989; Zhang *et al*.,1991). In humans, the metabolism of deltamethrin occurs by the hydrolysis of the ester groups (Chauhan *et al*.,1986; Barlow *et al*.,2001).In rodents, the metabolism of deltamethrin occurs by the cleavage of the ester link to produce acid and alcohol moieties, oxidation of various molecules, and conjugation with sulfuric acid, glycine, or glucuronic acid of the oxidation products. The cyano group of deltamethrin was converted to thiocyanate and excreted more slowly than other metabolites. Within 4 days, about 21% of metabolites were excreted in the urine and feces of mice, and 20% of thiocyanate was still retained in the skin and stomach for 8 days. (Erguven and Emel, 2019), results show that phytoremediation, where microorganisms and plants jointly detoxify and degrade pesticides is the best method for herbicide degradation. Alfalfa, maize, and soybean as crop remediators, and *Sphingomonas melonis, Sphingomonas aquatilis*, and *Bacillus subtilis* are the deltamethrin-degrading bacteria.Zhu *et al*.,(2020) illustrated the twelve pathways of deltamethrin photodegradation:- isomerization, ester hydrolysis, ester bond cleavage, c-o bond cleavage, 3,3-dimethyl acrylate formation, double bond break, C1-C3 bond cleavage in cyclopropyl, reductive dehalogenation,

decarboxylation,nucleophilic reagents attack, cyano hydrolysis and halogenated hydrocarbon hydrolysis.

2.7.3 Remediation methods of Deltamethrin

Due to its continued use, deltamethrin is detected in many environmental matrices, especially in soil and water where it can exhibit toxic effects on target and non-target organisms. Deltamethrin as a hydrophobic compound has low mobility in soils. As a result, this causes strong sorption to soil organic matter and limits its leaching into groundwater (Oudou and Hansen, 2002). Deltamethrin exhibits chemical and photochemical isomerization (Maguire, 1990).Erstfeld, (1999) determined that deltamethrin degraded with a half-life of 8 to 48 hours to decamethric acid. Caquet *et al*., (1992) observed that deltamethrin persisted in water only for 96 hours.

Deltamethrin undergoes various dissipation processes in soil and is degraded by hydrolysis, photolysis, and microbial activity. Depending on soil type and oxygen availability the half-life ranged from 11 to 72 days. (Elliot,1989; WHO, 1990). The bioavailability of insecticides is controlled by two major factors, clay content and organic matter in the soil (Zhang *et al*., 2007; Gu *et al*., 2008; Munoz-leoz *et al.,*2009). Deltamethrin persists for long periods in soils due to its high lipophilic nature (Oudou and Hansen, 2002). Khan *et al* (1988) reported that deltamethrin persists in soil for 40 months. Ismail, (2015) and Urkude and Kochhar, (2015) reported that under aerobic laboratory conditions the half-lives of deltamethrin in sandy loam or silt loam soil ranges from 11-72 days and ranges from 31-36 days in anaerobic soil conditions. He also found that it is not susceptible to photo-oxidation and is more persistent in soils with a high clay or organic matter content. The sorption of four pyrethroids in soil followed the order Lambda-cyhalothrin>Deltamethrin> Cypermethrin>Fenvalerate. The cumulative leaching of deltamethrin was small in soils (Zhu and Selim, 2002).

Degradation of deltamethrin in the soil is not only influenced by the microbes but also depends on the type of soil, soil organic matter, and soil texture. Gu *et al*., (2008) reported that the lower the content of clay and organic carbon in soil higher the degradation of deltamethrin. In contrast, Cycon *et al*., (2014) found that the highest dissipation of deltamethrin in soils is characterized by the highest organic matter. Ismail *et al*., (2013) demonstrated that deltamethrin possess a stronger affinity to the

silt clay soil and shows less mobility. Organic matter and clay contents are the major factors controlling insecticides bioavailability (Zhang *et al*., 2007, Gu *et al*., 2008; Munoz Leoz *et al*., 2009). Deltamethrin persists for long periods in soils due to its high lipophilic properties (Oudou and Hansen, 2002). Sandy soils with low content of organic matter and clay fraction show a higher ratio of deltamethrin dissipation (Cycon *et al.,* 2014). The degradation of deltamethrin was faster under aerobic conditions and the presence of nitrate had an inhibitory effect on their degradation. Bacterial and actinomycetes populations increases in soil containing deltamethrin. Wheat straw biochar amended soil increased the sorption of deltamethrin of soil to 3% and decreased the half-life (Palangi *et al*., 2021).

2.7.4 Deltamethrin Toxicity

Deltamethrin causes varying degrees of toxicity (Lu *et al*.,2019) and varies according to route of exposure. Deltamethrin shows high pyrethroid potency in insects followed by cyfluthrin, cypermethrin, flucythrinate, fenvalerate, and permethrin. In *Allium cepacium* the mitotic index is reduced due to deltamethrin toxicity. Widespread usage of deltamethrin pollutes water and food and is toxic to humans (Barlow *et al*.,2001). The toxicity levels of deltamethrin are it causes neuroproductive toxicity (Hintzen *et al*.,2009), cardiotoxicity (Haverinen and Vornanen, 2016) disrupts the endocrine system of non-target creatures, and causes chronic diseases (Abdel-Rahman, 2005). Deltamethrin acts as a human carcinogen (Rani *et al*.,2013).

Research studies report that oxidative stress, Reactive Oxygen Species, and Reactive Nitrogen Species generation play a key role in deltamethrin's toxic effects (Rehman *et al*., 2006; Muller *et al*., 2008; Romero *et al*., 2015).it decreased the feeding rates in red flour beetle (Ishaaya *et al*.,1983). Deltamethrin induces cell death and ROS production in rat primary hepatocytes (Arora *et al*.,2016). The exposure to low and high doses of deltamethrin for 16 weeks resulted in the formation of oxygen free radicals and hepatic toxicity in rat liver (Tuzmen *et al*.,2008). Li *et al*., (2007) found that the ROS production in PC12 cells was increased at a deltamethrin concentration of 10µM and resulted in early apoptosis of murine thymocytes. The treatment of swiss mice with deltamethrin showed changes in degenerative patterns in the liver and kidneys (Tos-Luty *et al*.,2001). Ding *et al*., (2017) reported that the deltamethrin concentration of 40mg/kg body weight caused dose-dependent neurotoxicity and liver

dysfunction in rats. It also resulted in elevated levels of reactive oxygen species. Experiments done in rats shows that deltamethrin exposure causes an increase in sperm abnormalities (Sharma *et al*., 2014) and nephrotoxicity. The immunotoxicity caused by deltamethrin in rats resulted in apoptosis of thymocytes and splenocytes (Guardiola *et al*.,2014; Kumar *et al*.,2015). Anadon *et al*., (1996) reported that the accumulation of deltamethrin metabolites increases neurotoxicity. The in vivo tests conducted by Eriksson and Fredriksson, (1991), and El-Gohary *et al*.,(1999) on gene mutations in mice resulted in micronucleus formation, chromosomal aberrations in the bone marrow, and abnormal sperm morphology. The overall evaluation reports that deltamethrin is not considered a strong mutagenic or clastogenic compound (Gupta, 2016). Pitzer *et al.,* (2021) figured out that long-term exposure to deltamethrin in mice and rats causes reductions in the dopamine transporter and the dopamine D1 receptor. Yang *et al*., (2022) observed that chronic exposure to deltamethrin decreases the protein expression of B-cell lymphoma genes in quail and causes cardiomyocyte inflammation and apoptosis. The survival ability of *Callibaetis radiatus* was affected by deltamethrin toxicity (Gutierrez *et al*.,2016). Arbuscular mycorrhizal fungi growth was decreased by deltamethrin (Rivera-Becerril *et al*.,2017).

Deltamethrin is also toxic to beneficial invertebrate species. Van dame *et al*., (1995) found that forager honeybees exposed to 2.5ng deltamethrin exhibited alternations in flight patterns and homing abilities. Decourtye *et al.,* (2005) reported that it reduces the proportion of bees exhibiting learned orientation towards an odour stimulus and causes sublethal effects in honeybees. Deltamethrin reduced the egg production, and capping rate, and extended the egg stage in honeybees (Dai *et al*., 2010). It causes parasitoid repellency and decreases female offspring wheat aphid parasitoids (Longley and Jepson, 1996). Plant-dwelling insects were more exposed to pyrethroid residues longer than soil-dwelling invertebrates because pyrethroids are more persistent on leaves (Wiles and Jepson, 1994). Kwong, (2002) studied the persistence of deltamethrin in an oil palm agroecosystem. It Adversely affects the growth of *Hyalella azteca* (Amweg *et al*.,2005). It is toxic to non-target organisms (Strachan and Kennedy, 2021). In mammals, pyrethroids cause two distinct syndromes and deltamethrin comes under CS (Choreoathetosis and Salivation) syndrome (Verschoyle and Aldridge, 1980).

Among pyrethroids, deltamethrin is the most toxic insecticide to aquatic organisms (Mueller *et al*., 1990; Tandon *et al*., 2005). Deltamethrin increased antioxidants in gills of freshwater fish Channa punctatus Bloch (Sayeed *et al*., 2003). It causes toxicity in fishes by blocking the Na channels of nerve filaments resulting in the lengthening of the depolarization phase and it also affects the GABA receptors in the nerve filaments (Eshleman and Murray, 1991; Moid *et al*., 2012; Prusty *et al*., 2015). Deltamethrin induces oxidative stress and histopathological changes in fishes (Yildrim *et al*.,2006; Yang *et al*.,2020). Pimpao *et al*., (2007) found that deltamethrin increases leucocytosis and increases erythrocytes in *Ancistrus multispinis*. It also affects the metabolizing and immune system of *A. multispinis*. It induces acute toxicity in the fry rainbow trout (Ural and Saglam 2005). Souza *et al*., (2020) reported that deltamethrin is highly toxic to most Amazonian freshwater fishes. The low water temperature helps to relieve the impacts of deltamethrin toxicity in fishes (Gewaily *et al*., 2021). It induces body spasms and uncontrolled swimming in young Zebrafish (Sharma and Badre, 2013; DeMicco *et al*., 2010). Velisek *et al.,* (2007) assessed the effect of deltamethrin on rainbow trout. The rainbow trout showed very low plasma glucose, alanine transferase, and cholinesterase as the result of deltamethrin toxicity. A lot of deltamethrin toxic studies were done on aquatic organisms. It is toxic to *Catla catla* (Vani *et al*., 2011), affects the mortality rate of young mirror carp (Calta and Ural, 2004), and toxicity on tissues of Nile tilapia fingerlings (Koprucu *et al*.,2006; Yildrim *et al.,*2006), toxic to sword fishtail (Khalili *et al.,* 2012) and chromosomal damage in fish (Marques *et al*., 2014). Simsek *et al*., (2008) studied the effects of deltamethrin on antioxidant stress and oxidative stress in the digestive gland and gill of freshwater mussels, which resulted in lipid peroxidation. Felten *et al*., (2020) found that the sensitivity of Daphna magna to deltamethrin was enhanced by microplastics.

Humans are exposed to deltamethrin through direct contact with the vapor, inhalation, epidermal contact, and ingestion. Occupational exposure is the root cause of human poisonings. The poisonings from occupational exposure involve mainly skin symptoms like burning and paraesthesia (Barlow *et al*.,2001). The other symptoms are severe headache, dizziness, fatigue, nausea and anorexia, muscle fasciculation, and convulsions. Ruzo *et al*., (1978) reported that two of the occupational cases died of convulsions. Deltamethrin toxicity leads to neurodegenerative and cerebrovascular disorders (Mani *et al*., 2017) and deltamethrin alters the signalling pathways of the

immune system. *Spirulina plantensis* protects male Wistar albino rats against deltamethrin toxicity by normalizing the elevated serum levels of uric acid, urea, and creatinine (Abdel Daim *et al*.,2013). The extracts of plants such as garlic, *Trigonella foenum graecum, Globularia alypum*, olive oil, and *Artemisia campestris* oil have protective effects against deltamethrin toxicity in the kidneys and liver. Pradhan *et al*.,(2011) revealed that the combination products of deltamethrin and triazophos will not lead to any residual toxicity plan. The studies on deltamethrin removal from vegetables was experimented by scientists. Pre-washing, preheating reduces the residues in tomato paste (Uysal *et al*.,2006) and in vegetables (Randhawa *et al*.,2008). Researchers developed a monoclonal Antibody based Immunoassay for deltamethrin detection.

2.7.5 Deltamethrin – Mode of Action

Deltamethrin poisoning occurs by cuticular penetration or by oral uptake (Ruzo *et al.,*1978; Anand *et al*.,2006). Sattelle and Yamamoto, (1988) studies show that deltamethrin poisoning lasts more than a few hours and causes irreversible damage to the central nervous system. Deltamethrin acts as a neurotoxin. The mode of action of deltamethrin mainly depends on the presence of an α-cyano group. This alpha cyano group induces the inhibition of the activation of the sodium channel gate and this leads to the increased permeability of the nerve to sodium thus resulting in the blockage of nerve conduction. Deltamethrin causes the phosphorylation of calcium and chloride channels in the target pests (Burr and David, 2004). Deltamethrin action is central and originates in higher nerve centers of the brain (Spencer,1981; Shrivastava *et al*.,2011;).

2.7.6 Deltamethrin – Environmental Fate

In soils, the significant degradation pathway of deltamethrin is photodegradation. Photodegradation takes place in three steps: - debromination, photoisomerization and ester bond cleavage (Maloney *et al*.,1988; Ferandez -Alvarez *et al.,*2007). Liu *et al*., (2010) found that photodegradation follows first-order kinetics with the production 3-phenoxybenzaldehyde and 3-phenoxybenzoic acid.

2.8 Propargite – a sulfite ester acaricide

Propargite was one of the most important second-generation acaricides, developed during the 1960s and early 1970s. Propargite (2-[4-(1,1- Dimethylethyl) phenoxy] cyclohexyl 2-propynyl sulfite) is a non-systemic acaricide. It is being applied for controlling a variety of phytophagous mites on many crops including cotton, vines, fruit trees, vegetables, hops, and nuts, etc. (Royal society of chemistry, 1987). In tea plantations, it is used for controlling the red spider mite. WHO classified propargite as slightly hazardous (WHO, 1999). It is a toxic air contaminant.

Propargite has introduced almost 50 years ago and it has significant acaricidal effects against mites in ornamentals and orchards (Koh *et al*.,2009; Luo *et al.,*2014; Mohammedzadeh *et al*.,2014). The maximum residue level (MRL)for propargite was 50mg/kg & 10mg/kg in tea and citrus fruits respectively (EFSA,2018).

Table 2.12 : Chemical and Physical properties of Propargite

Chemical formula	$C_{19}H_{26}O_4S$
Molecular mass	350.47
Pesticide type	Acaricide, Insecticide
Substance groups	Sulfite ester insecticide, sulfite ester insecticide
Minimum active purity	870g/kg
Substance origin	Synthetic
Mode of action	Non-systemic with contact action
IUPAC name	2-(4-tert-butylphenoxy)cyclohexylprop-2-ynylsulphite
Physical state	Dark yellow-brown viscous liquid
Density	1.113
Boiling point	Decomposes before boiling
Solubility in water at 20^oC	0.215mg/L
Solubility in organic solvents	Hexane=toluene=dichloromethane=acetone

2.8.1 Propargite-Mode of Action and Potency

Rezaei *et al*., (2007) Studied the effects of propargite on biological control agent *C. camea* (Stephens)by using the IOBC approach and life table response experiments (LTRES) propargite had harmful effects on fecundity & was slightly harmful. Liang *et al*., (2018) Evaluated the cotoxic effects of trifenzate and propargite against

Tetranychus urticae Koch and resulted in an increase in joint inhibition effect. (Reddy *et al*., 2013). Propargite was the inhibitor of ATPase (Kadir and Knowles 1991). Luo *et al*., (2014) defined the specific (GST)glutathione S-transferases genes involved in propargite resistance of carmine spider mites in China. *Aedes aegypti* was least susceptible to propargite (Pridgeon *et al*., 2008).

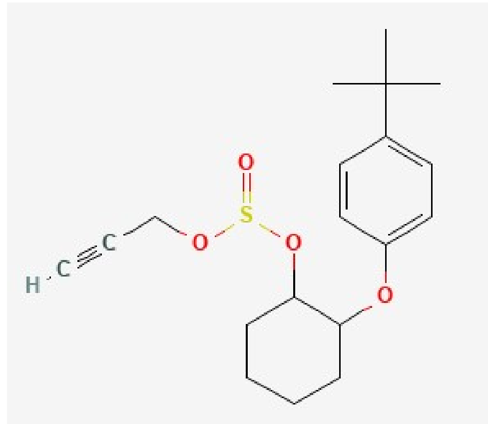


Figure 2.16 Chemical structure of Propargite (ChemPub)

Propargite residues in greenhouse tomatoes are reduced slowly with time (Aplada - Sarlis *et al*., 1994). Sherwani *et al*., (2019) observed that double the concentration of propargite increased the mortality rate in *Malus domestica Borkh*. Alinejad *et al*., (2020) analyzed the sublethal effects of propargite on *Amblyseius swirskii.* It affected the total life span and longevity of treated mites. Luo *et al*., (2018) attempted to identify the specific ATPase genes involved in propargite resistance in *T.uinnabarinus* and resulted in the expression of 3 genes:Tc ATPsynu-2, TcATpsyn Fo-2&TcATpsyn Fo-4. Wang *et al*., (2018) studied the resistance of pacific spider mite to propargite and showed 100% mortality of adult females in populations. (Vostrel, 2008)studied the effectiveness of propargite against two-spotted spider mites which caused 100% mortality.

Propargite has the potential to affect the life cycle and fecundity of *Macrolophus pygmaeus*, which is a generalist predator and a biological control agent (Rahmani *et al*., 2021). Used for control of several phytophagous mites active on fruit trees, vegetables, cotton and many other crops. It inhibits magnesium-stimulated ATPase (Sherwani and Mukhtar, 2019) and has adverse effects of propargite several studies (Lira *et al*.,2015; Asadi *et al*.,2019; Alinejad *et al*.,2020). Ghamari *et al*., (2021) and Kavya *et al*., (2015) studied the combined effects of propargite along with

spirodidofen, extracts of shirazian thyme and rosemary on control of *Tetranychus Urticae*. Propargite was effective against the red spider mite (*Oligonychus coffeae*) the most destructive pest of tea (Banerjee *et al*., 2009)at low doses (Somnath Roy *et al*.,2012). Propargite functions as a respiration inhibitor by ATPase blocking in aquatic and terrestrial organisms (Sanchez Bayo, 2012).It is considered as the best control program of *T.urticae*, due to its more toxicity and faster reversion rate (Khadri *et al*., 2020).It control –citrus rust mite, *Phyllocoptruta oleivora* (Rao *et al*.,2014) plays an important role in the control of *Brevipalpus phoenicis*. Propargite residues in plant is hydrolyzed to form TBPC, TBPC-Diol or to aliphatic oxidation (HOMe-TPBC) (EFSA, 2013). Insecticides combination of propargite, spirodiclofen and cyfhimetofen had no interference in the control of pest mite *Brevipalpus* (Della *et al*.,2019). The laboratory tests done by (Marshall and Pree, 1993) showed that toxicity of propargite it correlated with temperature, in the case of European Red Mite. Propargite showed good efficacy and mites mortality on *Tetranychus* infesting Piper beetle (Srinivasa and Pushpa, 2016). Propargite at a concentration of 433.32 ppm result in LC50 values for eggs and causes ovicidal toxicity (Roy *et al*., 2018). Toxicity effects varies according to the biological characteristics of mites. Propargite is safer for honeybees while using at flowering period (Rasuli *et al*., 2017) and effective against chilli yellow mite (Tudu *et al*., 2017). Budianto *et al*., (2021)stated that the population of predatory mite *Amblyseius deleoni* was decreased due to continuous use of propargite, fungicide and neem seed extracts in tea populations. Propargite belongs to phenoxy group causes damage to the respiratory system. Through "residual killing action" it inhibits cholinesterase in acetylcholine hydrolysis into choline and acetate. The toxicity of propargite to adult female *Phytoseiulus persimillis* was positively correlated with temperature (Poletti and Omoto, 2012). Propargite inhibits Mg^{2+} ATPase preparations from bulb mites (Kadir and Charles, 1991). Propargite reduced the population of rice leaf mite by 93-96% after 14 days of spray treatment (Shankar *et al.,*2019). Propargite induces pancreatic B-cell death (Zhon *et al*.,2018). Gupta *et al*., (2020) suggested that propargite contributes 6.75% for the management of yellow mite. Tulgar, (2019) studied the accumulation of propargite in muscle tissues of carp. Stara *et al*., (2011) evaluated the side effects of propargite on natural enemies or predators of the pest.

2.8.2 Propargite- Toxicity

The residues of propargite persisted in red chili for five days and the half-life was 0.63 days (Varghese *et al*., 2011). Propargite is identified as medium-risk pesticide under normal growing conditions (Ashley *et al*., 2006). In Japan propargite residues is higher in land with chemical fertilizer treatment than in organic fertilizer treatment (Kai and Adhikari, 2021).It shows 90% mortality rates against *Tetranychus urticae Koch* (Kumari *et al*., 2017). It is highly toxic to fish and crustaceans (Sanchez-Bayo,2019). Propargite is an irritant in humans and causes dermatitis in field workers. In soil, it is immobile and moderately persistent. The persistence rate varies in relation to the environmental pH (Sarkar *et al*., 2010).

Propargite affected the germination of primary conidia of the mite pathogenic fungus *Neozygites floridana* (Wekesa *et al*.,2009). Propargite increased the level of bilirubin uric and, creatinine, Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) and decrease the level of cholesterol and alkaline phosphatase (AP)in albino male rats. Through inhalation and dermal routes, propargite enters the human body. The residues of propargite were detected in bee pollen, which poses a risk to human health (Zafeiraki *et al*.,2022). Banerjee *et al*., (2009) found that the propargite dissipation follows I^{st} order reaction kinetics with a half-life of 2.38-3.04 days for okra and 5.63-7.06 days for brinjal.

Mageswari *et al*., (2018) analyzed the alternations in histopathological parameters of freshwater fish *Channa striatus* to the exposure of propargite. It resulted in a reduction of RBC count, hemoglobin value, packed cell volume, and, mean corpuscular hemoglobin (MCH). Ma J *et al*., (2008) showed the toxic effects of propargite on three cyanobacteria and five green algae. Propargite was the least toxic to *Chironomusus dilutes* and *Hyalella azteca* (Ding *et al*., 2011). The sublethal doses of propargite negatively affected the adult longevity, survival fecundity, fertility, hatch rate and sex ratio of important ectoparasitoid wasp, *Habrobracon hebetorsay* (Asadi *et al*., 2019). It is toxic to predatory coccinellids (Akhtar, 2022).

2.8.3 Bioremediation of Propargite

The microbial respiration and microbial biomass were inhibited by the heavy and long-term use of propargite in Pakistan (Hussain *et al*., 2001). Chaiya *et al*., (2021) isolated actinobacteria strains like *Streptomyces* and *Amycolatopsis* (GLM-2) which

tolerate the insecticide propargite. Sarkar *et al*., (2010)isolated propargite degrading bacteria *Pseudomonas putida* from tea rhizosphere about 71.9% of degradation was exhibited by this species.

2.9 Fenpyroximate – An Acaricide

Fenpyroximate is an novel acaricide discovered in 1985 at Japan by Nihon Noyaku and commercialized in 1991, belongs to phenoxy pyrazole group. It comes under the category of inhibitors or uncouplers of mitochondrial respiration. It is selective and active against important phytophagous mites such as Tetrabychidae, Eriophyidae, and Tarsonemidae by inhibiting the mitochondrial complex I. The key metabolites of fenpyroximate are (E) -4-((1,3-dimethyl-5-phenocypyrazole-4-yl) methyleneaminooxy-methyl) benzoic acid, 1,3-dimethyl-5-phenoxypyrazole-4-carbonitrile and 1,3-dimethyl-5-phenoxypyrazole-4-carboxylic acid. Fenpyroximate was found to be the best acaricide available in controlling chilli yellow mite (Biswas *et al*., 2009). It is a chemical with knockdown activities and an acaricidal effect on phytophagous mites belonging to Tetranychidae family due to the inhibition of NADH: ubiquinone oxidoreductase (complex I), the mitochondrial respiratory chain enzyme (Shiraishi *et al*.,2012). Fenpyroximate belongs to the group of mitochondrial complex I electron transport inhibitors and chemical classification of METI acaricides and insecticides.

Table 2.13 Properties of Fenpyroximate

Chemical formula	$C_{2}4H_{2}7N_{3}O_{4}$
Molecular mass	421.49
Pesticide type	Acaricide, insecticide
Substance groups	Pyrazolium
Minimum active purity	960g/kg
Substance origin	Synthetic
Mode of action	Mitochondrial complex I electron transport inhibitor, contact inhibition
IUPAC	Tert-butyl[E]-α-(1,3-dimethyl-5-phenoxypyrazol-4-ylmethyleneaminooxyl)-p-toluate
Physical state	White crystalline powder
Melting point	102° C
Boiling point	Decomposes before boiling
Density	1.25g/ml
Solubility in water at $20^{o}C$	0.021
Solubility in organic solvents at $20^{o}C$	Xylene>acetone>ethanol>Methanol.

2.9.1 Fenpyroximate – Mode Of Action and Toxicity

Its mode of action is the inhibition of mitochondrial transport at the NADH coenzyme Q reductase site of complex I (Motoba *et al*.,1992). It doesn't have any clear toxic effect on other arthropods such as insects, and soil-living mites (Motoba *et al*.,1992; Kim *et al*.,2004).

2.10 Spiromesifen- Tetronic acid derivative acaricide

Spiromesifen[3-2,4,6-trimethylphenyl)-4-(3,3-dimethylbutyl-carbonyloxy)-5spirocyclo-pentyl-3-dihydrofuranon-2 is a selective, tetronic acid derivative insecticide or miticide/acaricide belongs to the chemical class of spirocyclic phenyl susbstituted tetronic acid and ketoenols used on fruit, vegetable and ornamentals. It controls pests like whiteflies, and mites (including spider mites, grass mites, and broad mites) (Nauen *et al*., 2003; Kontseclalov *et al*., 2009). It is discovered by Bayer Crop Sciences during the 1990s. It has a new mode of action with insect growth regulator (IGR)- like properties that interfere with lipid biosynthesis, particularly inhibition of acetyl CoA – carboxylase and lipid metabolism enzyme, affecting the development of growth stages of younger insects and affecting the fecundity of adults (Dekeyser, 2005).

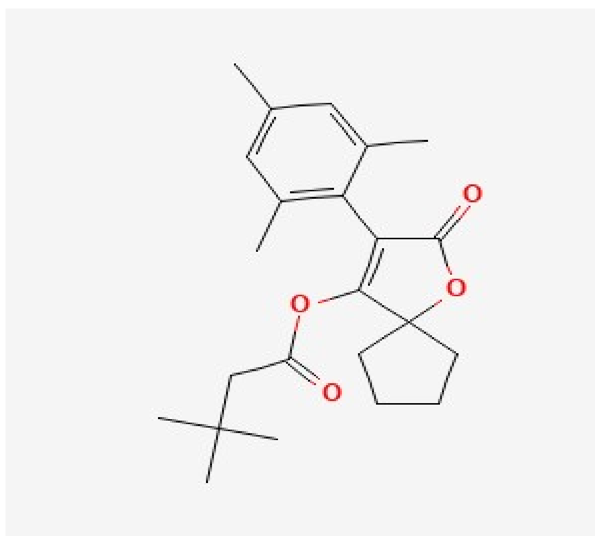


Figure 2.17 Chemical structure of Spiromesifen (ChemPub)

Table 2.14 Chemical and Physical properties of Spiromesifen

Chemical formula	$C_{23}H_{30}O_4$
Molecular mass	370.48
Pesticide type	Insecticide, acaricide
Substance groups	Tetronic acid insecticide, tetronic acid acaricide
Minimum active purity	965g/kg
Substance origin	Synthetic
Mode of action	Non-systemic, inhibitor of acetyl CoA carboxylase
IUPAC name	3-mesityl-2-oxo-1-oxaspiro[4,4]non-3-en-4-yl3,3-dimethylbutyrate
Physical state	Colourless crystals
Melting point	98
Boiling point	Decomposes before boiling
Solubility in water at 20°C	0.13
Solubility in organic solvents	Xylene=Acetone=Ethyl acetate >n-heptane
Density	1.13g/ml

New acaricides like spiromesifen can be used for effective management of the mites and it is compatible with recommended concentrations of *B.thuringiensis* (Lucas *et al*.,2014). The activity of spiromesifen is less potent than other insecticides against the most abundant and investigated mosquito species, *Culiesta longiareolata* (Bouabida *et al*.,2017). It comes under the category of growth-inhibiting acaricides. The main energy source in mite eggs are lipids. As a result, the disruption of lipid utilization will lead to toxic effects in mites. it is a potential acaricide to control sheep scab mite, *P.ovis* (Dunn *et al*.,2016), and whitefly, *Bemisia tabaci* in tomato (Abhishek *et al*.,2021). Spiromesifen was the least effective against *E.vermiculata* (Hussein and Sabry, 2019).

2.10.1 Degradation of Spiromesifen

In the case of degradation studies of spiromesifen it follows first-order kinetics in soil and it shows an increased rate of degradation in composite soil samples than in normal soil samples (Mate *et al*.,2015). The half-life of spiromesifen is 1.94 days in

okra fruit with a 98% of dissipation rate after 15 days of spraying (Muppala and Peddi, 2020). Photodegradation is the major route for dissipation of spiromesifen in the tomato leaves and in the case of fruits it is the combination of photodegradation and dilution rate due to fruit growth (Siddamallaiah *et al*.,2017). The degradation rate of spiromesifen was faster under UV light conditions than under sunlight (Mate *et al*.,2015). Spiromesifen exhibits very low mobility in plants (Weber, 2005). Spiromesifen-enol is the major metabolite of spiromesifen, which is formed by the hydrolysis of the parent compound.

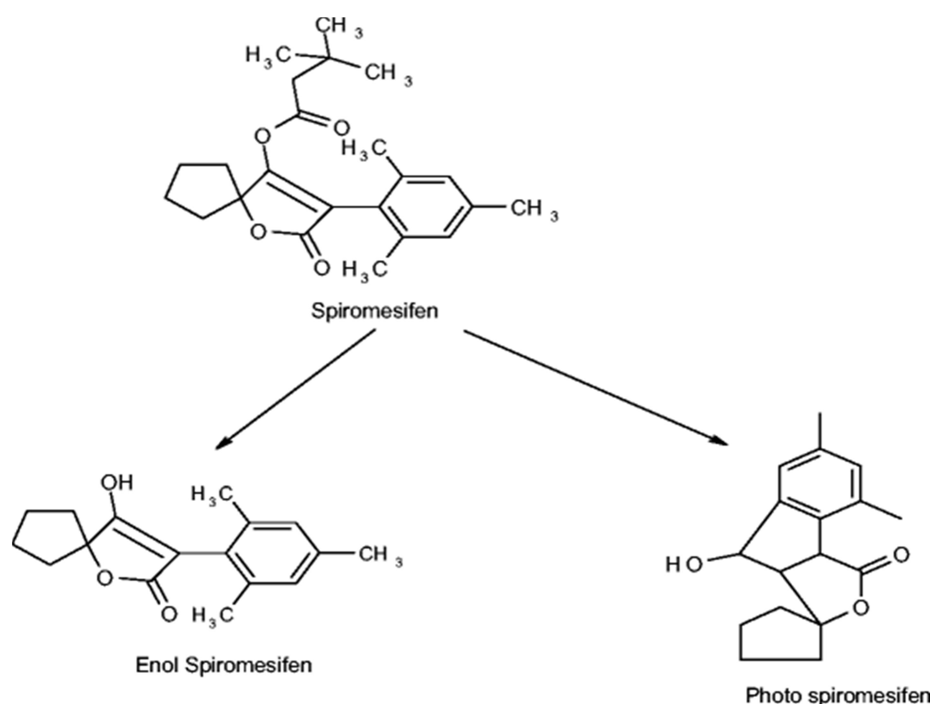


Figure 2.18: Spiromesifen metabolites Enol-spiromesifen and Photo-spiromesifen (Weber, 2005)

2.10.2 Spiromesifen – Toxicity

Spiromesifen is toxic at various levels. Karakayali *et al*., (2021) studied the moderate toxicity of spiromesifen in mouse neuroblastoma cells in vitro using a neurotoxicity screening test. At high concentrations, it caused neurite inhibition, decreased proliferation, and reduced the viability of cultured neurons. It was toxic for rat hepatocytes at 3mg/kg. Fang *et al*., (2022) showed that the metabolite, enol-spiromesifen was more persistent in soil and *E.fetida* than spiromesifen. It damages the *E.fetida* epidermis and enhanced lysosomal and phagosomal activities. It is a growth interrupter of mosquito species, *Culiesta longiareolata* Aitken and *Culex*

pipiens (Bouabida *et al*., 2017). Spiromesifen affected the lipid biosynthesis in the Zebrafish embryos, decreased the cholesterol contents and is most toxic to Zebrafish (Zhang *et al*.,2019). The pesticide mixtures including spiromesifen adversely affected and altered the liver and kidney biochemical markers of male Wistar rats. The two major metabolites are enol-spiromesifen and photo-spiromesifen. Spiromesifen and its metabolites were detected in edible fungi (Tian *et al*., 2021). Spiromesifen reduced the sex ratio, and fecundity of the model organism *D.melanogster*. It also affected the sexual behavior of the organism (Kissoum *et al*., 2020). It increases the LDH activity in *D.melanogster* by disturbing the energy metabolism and inducing chemical stress (Kissoum and Soltani, 2016). It induced a high level of immobilization in Salmon louse, *Lepeophtheirus salmonis kroyer* (Aaen and Horsberg, 2016). Spiromesifen caused delayed effects on morphometric measurements and the protein content of ovaries in *D.melanogaster* (Kissoum *et al*., 2020).

2.11 Thiamethoxam – Neonicotinoid Insecticide

Thiamethoxam (TMX) is a systemic, second-generation neonicotinoid insecticide that belongs to the subclass of nitroguanidine group and thianicotinyl subclass (Maientisch *et al*.,2001).It is a chlorothiazolylmethyl insecticide. It is marketed in the tradename Actara and Cruiser. Actara is used for foliar drench and soil treatment, whereas cruiser is for seed treatment. Thiamethoxam was developed by Ciba Crop Protection in 1996, first registered in New Zealand, and marketed by Syngenta in 1998. Thiamethoxam is the result of combination of two compounds 4-nitroimino-1,3,5-oxadiazinane and 2-chloro-5-thiazolyl moiety with high insecticidal activity. It is highly effective and has control of a wide variety of sucking insect pests such as aphids, whiteflies, leafhopper, and coleopteran pests, on a variety of crops including barley, cotton, sorghum, wheat, canola, and corn. Thiamethoxam also controls important vectors of plant virus diseases, by suppressing the secondary spread of viruses in various crops Maienfisch *et al*., (2001) found the advantages of thiamethoxam such as low use rates, flexible application methods, excellent efficacy, long-lasting residual activity, and favorable safety profile. Thiamethoxam holds registration for 115 crop uses in at least 64 countries (Elbert *et al*.,2008; Jeschke *et al*.,2011). Thiamethoxam is one of the fastest-growing neonicotinoids on the insecticide market (Pandey *et al*.,2009; Dance *et al.,* 2017).

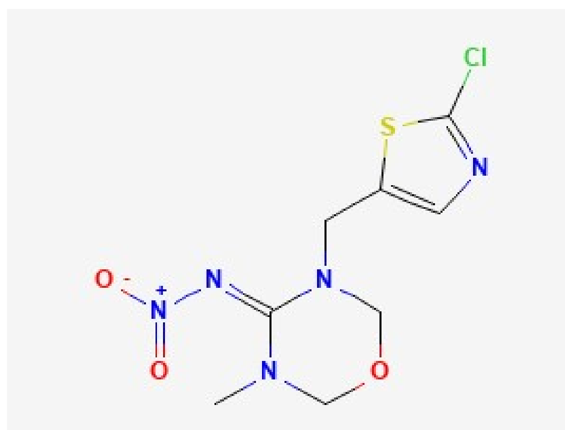


Figure 2.19 Chemical structure of Thiamethoxam (ChemPub)

Table 2.15 Chemical and Physical properties of Thiamethoxam

Chemical formula	$C_8H_{10}ClN_5O_3S$
Molecular mass	291.71
Pesticide type	Insecticide
Substance groups	Neonicotinoid, nitroguanidine, thiazole, insecticide
Minimum active purity	980g/kg
Substance origin	Synthetic
Mode of action	Broad spectrum, nAChR competitive modulator
IUPAC name	(E2)-3-[2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nito)amine
Physical state	Crystalline powder
Melting point	139.1°C
Boiling point	Decomposes before boiling
Density	1.57g/ml
Solubility in water at 20°C	4100mg/L
Solubility in organic solvents	Acetone>Ethylacetate>Toluene>Hexane.

2.11.1 Mode of Action and Potency of Thiamethoxam

It is a neurotoxin, that acts as an agonist of the nicotinic acetylcholine receptors (nAChR). It has a similar mode of action to nicotine, like linking the synapses of the nervous connections to the acetylcholine receptors. Thiamethoxam has both contact

and systemic activity against target pests, because, dissolved thiamethoxam is taken up with the soil water into the plant and transported acropetally into the xylem (Tomlin, 2003). Thiamethoxam acts as a feeding deterrent and through the xylem, it is transported to all parts of the plants. Thiamethoxam becomes active in the stomach of insects by direct contact and paralyzes the insect by interfering with the nerve cells. In crops, it induces the expression of target functional proteins involved in the stress defense mechanisms allowing them to cope under growing conditions. The high insecticidal activity of thiamethoxam is due to the presence of the 4-nitroimino1-3-5-oxadiazinan group attached to halogenated thianicotinyl moiety. Thiamethoxam is used for seed treatment. It promoted the linear development of roots, increased protein content, increased shoot dry matter, and acts as a bio-activator in crops (Macedo *et al.*,2011).

Thiamethoxam has productive effects on plant health, such as increasing plant vigor, crop yields, and increasing biotic or abiotic stress on the tolerance of plants (Calafiori and Barbieri, 2006; Horii *et al*., 2007). Ford *et al*., (2010) stated that thiamethoxam penetrated into the plant cells and induced specific functional proteins involved in various stress defense mechanisms. Macedo and Castro, (2011) suggested that thiamethoxam has a role as a growth regulator by altering plant physiology and shows potential for increasing crop production. It also enhanced the production EPA and other substances by PGPR (Plant Growth promoting Rhizosphere).

2.11.2 Toxicity of Thiamethoxam

Research studies on the toxicological effects of thiamethoxam were done by several researchers. Gajger *et al., (*2017) reported that thiamethoxam reduced the honeybee body weight and ovary weight. It also affected the sperm counts of honeybees. Delgarde and leferre, (2002) found that it is toxic to honeybees, harmful to soil and aquatic organisms, and also exhibits termiticidal properties. it poses antifeedant action (Acda, 2007). Neonicotinoid pesticides including thiamethoxam were toxic to embryos of mice and rabbits (Babelova *et al*., 2017). The toxicity Study of thiamethoxam done on H295R human cells resulted in the inhibition of 16α-hydroxylation of foetal DHEA (Caron – Beaudoin *et al*., 2017). The European Siberian carabid *Platynus assimilis* insects expressed hyperactivity (Tooming *et al*., 2017). Thiamethoxam leads to premature death and decreases the survival capacity of

bumblebees (Ellis *et al*., 2017). Thiamethoxam affected the flight ability and phototropism, and seriously affected the gene expression (Christen *et al*.,2018) of honeybees. Senyildiz *et al*., (2018) found that thiamethoxam causes cytotoxicity and DNA damage to HepG2 and SH-SY5Y cells. It acts as a carcinogen and endocrine disrupter in Mongolian Racerunner (Wang *et al*., 2019). At sub-lethal concentrations thiamethoxam reduced the survival and growth of Mayfly (Bartlett *e*t *al*., 2018). In Chinese lizards, thiamethoxam increased the concentrations of acetylcholine in the brain and blood (Wang *et al*.,2019). It reduced the survival ability of freshwater amphipods (Bartlett *et al*.,2019) and is also toxic to farmland lizards (Wang *et al.,*2019). Thiamethoxam is a potential contaminant of surface and underground waters. Thiamethoxam affected the colonization of invertebrate populations in aquatic microcosms(Basley and Goulson, 2018). European Food Safety Authority (EFSA) summarised that thiamethoxam poses a high risk to non-target pests such as honeybees. In view of risk assessment European union partial, banned thiamethoxam in May 2013.

Kakamand *et al*., (2008) showed that thiamethoxam induced 90% mortality in honeybees. It reduced the homing ability and survival of foraging honeybees (Henry *et al*.,2012). Thiamethoxam caused impairment in the brain and midgut of honeybees and reduced their lifespan (Oliveira *et al*.,2014). It induced 50% mortality of honeybees within 3.5hours (Oliveira *et al*.,2009) and was extremely toxic to the Africanised honeybees (Laurino *et al*.,2011). It affected the homing flight of honeybees (Decourtye, 2010). The pupation and survival of honeybee larvae were affected at a sublethal concentration of thiamethoxam (Li *et al*.,2021). Thiamethoxam reduced the quality of oocytes in cattle and inhibited the metaphase I stages and CDC25 and CDC2 activity (Nie *et al*.,2019).

2.11.3 Metabolites of Thiamethoxam

Thiamethoxam is hydrolytically very stable at pH 5 and pH 7 and it is easily altered at pH 9. Only a few thiamethoxam degradation studies were done in microbial systems (Ford and John, 2008). They degrade thiamethoxam by the nitro reduction metabolic pathway and result in the formation of metabolites such as nitrosoguanidine/nitrosoamine, amino guanidine, desnitro/guanidine/imine and urea (Pandey *et al*.,2009). The main metabolite of thiamethoxam in the soil is clothianidin,

its highly persistent and toxic to insect pests whereas other degradation metabolites are non-insecticidally active. Two metabolites of thiamethoxam, N-nitrosoimino(=N-NO) and urea(=O) are reported by (Zhou *et al*.,2013) in the soil in China as a result of bacterial degradation with a retention time of 5.22 and 9. 21mins. Microsomal CYP450 is effective for the conversion of thiamethoxam to clothianidin. Thiamethoxam was also converted through the demethylation pathway. Several researchers (Karmakar *et al*.,2009) studied the metabolism of thiamethoxam in mice, insects, and a few plants such as cotton, spinach, and tomato. The metabolism or transformation of thiamethoxam follows three pathways, one by demethylation to desmethyl thiamethoxam, the second by nitro reduction to nitrosoimino, and at last one by transformation to imino and urea or cleavage of the oxadiazine ring to give clothianidin. Patil *et al*., (2021) identified the metabolite thiamethoxam urea along with nine degradation products. He also studied the degradation of thiamethoxam by ultrasound cavitation at a frequency of 20Khz and intensified the process by the addition of water, Fenton, and photo-Fenton reagent.

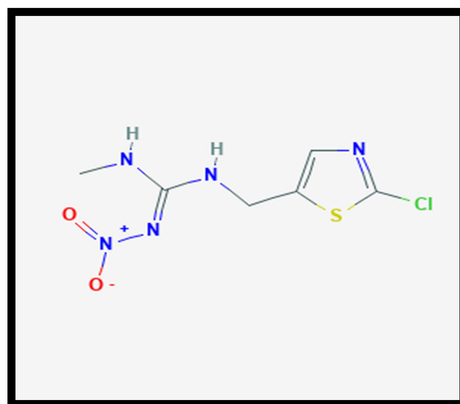


Figure 2.20 Chemical structure of Clothianidin (ChemPub)

2.11.4 Degradation of Thiamethoxam

Thiamethoxam is degraded and removed from the environment in several ways. The photolytic degradation of thiamethoxam was studied by researchers all over the world (Sojic *et al*.,2012; Mir *et al*.,2013; Yang *et al*.,2014). The combination of Fenton’s reagent with the biological oxidation process enhanced the thiamethoxam removal process (Gomez Herrero *et al*., 2019). The pesticide manual for thiamethoxam states that ‘Photolysis accelerates degradation in soil’. Yang *et al*., (2014) proved that the degradation products of thiamethoxam can be further mineralized into inorganic

substances such as CO_2 and H_2O. in biological degradation, soil polluted with thiamethoxam is remediated by green nano phytotechnology. The plants used were Marjoram and Moringa (Rady *et al*., 2019). Thiamethoxam will not be volatilized from soil or crops in significant amounts because of its low volatility. Trace elements of thiamethoxam in the air would be subjected to rapid oxidative degradation by hydroxyl radicals.

The data regarding the rate of degradation of thiamethoxam in the soil is inadequate. Microbial degradation is the most acceptable form of neonicotinoid remediation from the environment. The main pathway of thiamethoxam metabolism in the soil is microbial degradation (Gupta, 2008). The metabolism of thiamethoxam by the nitro reduction pathway will release nitrogen into the soil which influences the soil microbial structure.

Species that were capable to degrade thiamethoxam include *Bacillus subtilis* FZB24, *Bacillus amyloliquefaciens* IN937a, *Bacillus pumilus* SE34 (Myresiotis *et al*., 2012) *Ensifer adhaerens* TXM-23 (Zhou *et al*.,2013) *Bacillus aerophilus*, *Acinetobacter,* S*phingomonas* and *Pseudomonas putida* (Rana *et al.,*2015). *Acinetobacter* sp.,*Enterobacter.,Bacillus* sp., (Hegde *et al.,*2017). Zhou *et al*., 2013 recognized a soil enrichment culture comprised of members of the genera *Achromobacter*, A*gromyces*, *Ensifer*, *Mesoehizobium, Microbacterium*, and *Pseudoxanthomonas* to degrade 96% of thiamethoxam over 30 days. Pandey *et al*., (2009) identified three members of the *Pseudomonas* genus capable of reducing THM. Rodriguez Castillo *et al*., (2019) removed the neonicotinoid insecticides imidacloprid, thiamethoxam, and acetamiprid by a microbial consortium in a reactor scale. The bacterial species in the microbial consortium were *Paenibacillus, Rhodococcus, Microbacterium, Kocuria, Paraburkholderia* and *Pseudoacidovorax*, and a yeast strain closely related to the genus *Rhodotorula*. The bacterial species *Pseudomonas fluorescens, Pseudomonas putida*, and *Pseudomonas aeruginosa* potentially degraded thiamethoxam and used it as their sole carbon and nitrogen source (Zamule *et al*.,2021). Zhan *et al., (*2021) isolated an endophytic bacterial strain *Enterobacter cloacae* TMX-6 in rice plants and it removed 99% of thiamethoxam from a hydroponic medium within 21 days.

The areas of soils exposed to thiamethoxam will vary depending upon the application method. In the case of spray applications, the soil surface is the initial area exposed

followed by the upper soil layers. The half-life of thiamethoxam varies up to 400 days depending on different environmental conditions (Gupta *et al*., 2008) Due to its large half-life period major content of active neonicotinoid ingredients is dispersed into the environment leading to an environmental issue (Zamule *et al*., 2021). The half-life of thiamethoxam varied with moisture content ranging from 46 to 301 days (Gupta *et al*., 2008). Mortl *et al*., (2016) evaluated the mobility of thiamethoxam in three types of soils such as sand, clay, and loam soils, and stated that it is more mobile in sand soil than in loam and clayey soils. The adsorption and desorption of thiamethoxam in soils mainly depend on the soil's organic matter and cation exchange capacity (Han *et al*., 2019).

2.12 Microbial Diversity and Structure in Soil

Microbial diversity is a broad term, which comprises the number and relative abundance of distinct microbial species found in the soil, as well as the organization of the microbial community (Nannipieri *et al*., 2003). The type of species, its relative amount, contribution, and interrelationships between the different microbial functional groups forms the community structure (Harris and Steer, 2003). The soil microbial community is a result of more than 3.5 billion years of evolution and these communities are the most species-rich communities of terrestrial ecosystems.

Soil organisms are highly dynamic, which rapidly react to the environmental conditions (Winding *et al*., 2005; Moreira *et al*., 2006;). Soil organisms, especially microbiota plays an essential role in the stabilization of soil structure and fertility of soil, by regulating nutrient dynamics, acting as nutrient pool, energy transfer through soil food web and as sensitive indicators of soil fertility (Kandeler *et al*.,1996; Wardle *et al*., 2004). Microbial diversity and microbial functionality of soil is positively inter-related (Coleman Parkinson, 1991). Microorganisms determines the soil quality by maintain soil functions like soil structure formation, decomposition of organic matter, toxin removal, cycling of carbon, nitrogen, phosphorous and Sulphur, substrate utilization decontamination of soil different enzyme activities (Van elsas and Trevor, 1997).

The diversity of soil microorganisms is tremendous (Torsvik, Salte 1990). About 0.1% of the soil microorganisms are cultured and the metabolic rate is understood. Microbial communities are phenotypically and genotypically highly diverse. Hence,

they are difficult to characterize completely (Torsvik, 2002). Microbial diversity is large in topsoil because of the presence of a great variety of nutritional sources (Tiedje *et al*., 2001) and the bacterial population in soil top layers can go upto 10^9/g of soil (Torsvik and Ovreas, 2002). Most of the annual carbon and nutrient flux is mediated by soil organisms and its occurring in the top 5 – 10cm of the soil sample (Wolters, 1997). There are several practical difficulties in assessing soil microbial diversity because the link between species diversity and soil functioning remains poorly understood (Nannipieri *et al*., 2003). Counting methods, such as the plate count technique have been used widely to assess microbial diversity. The amount of culturable bacteria is increased by using several incubation parameters like nutritional media type, temperature, water potential and time (Nannipieri *et al*., 2002; De vries *et al*.,2006). Several microorganisms are identified in this way.

Microorganisms particularly pesticide degrading microbes are isolated through culture dependent methods, but these culture dependent methods are biased. To overcome the shortcomings of culture dependent methods, molecular biological techniques are increasingly used (Dunbar *et al*.,2000; Torsvik and Ovreas, 2002; Simonsen *et al*.,2006; Zhang *et al*., 2006). The diversity of soil microbial population can be assessed on the genotype level by total community DNA or RNA fingerprinting methods (Van elsas *et al*., 2006), DNA (Dilly *et al*., 2004; Mills *et al*., 2004). 16srRNA sequences reveal numerous uncultured microorganisms in a natural community (Ward *et al*.,1990).

2.13 Microbial degradation of pesticides in soils

Microorganisms have a major role in the degradation of synthetic chemicals in the soil. Microbial degradation or biodegradation is the main source of pesticide degradation in soils (Edgehill and Fin, 1983; Waldman and Adin,1998;). It is a natural process by which fungi, bacteria, and other microorganisms in soil have the unique ability to use or consume all-natural and synthetic compounds like xenobiotics as their sole carbon and energy source thus producing carbon dioxide, water, and methane (Alexander, 1981; Bangar *et al*., 2020). Microorganisms are highly efficient in the complete mineralization of several aliphatic, aromatic and heterocyclic compounds in the environment**.** It is a simple, inexpensive, and more environmentally friendly process to reduce pollution in soils (Diaz, 2004; Ye Jing *et al*., 2004). The great

versatility of microorganisms aids as a great advantage for this process. Microorganisms can grow and survive under high-stress concentrations of insecticides and toxic pollutants. Microbial degradation depends on various factors like the ability of microbes to produce requisite enzymes and ideal environmental conditions for the reactions to occur, sufficient microbial biomass, and the interaction between pollutants and the intercellular and extracellular enzymes. A variety of organic compounds are biodegraded (Klecka,1985; Wackett, 2002). Soil microbes play an important role in the recycling of plant nutrients, the maintenance of detoxification of noxious chemicals, and the control of plant pests and plant growth. (Brookes, 1995; Giller *et al*., 1998).

Research reports document the ability of soil microorganisms to degrade pesticides in the soil environment (Siddique *et al*., 2003; Kumar and Philip, 2006; Hussain *et al*., 2007). Pesticides influence on the environment is largely affected by the presence of microbes in the soil. Most of the pesticides are degraded and others are resistant. A diverse group of bacteria metabolize pesticides. *Alcaligenes, flavobacterium, pseudomonas, Rhodococcus* etc. are the identified bacterial strains which degrade the pesticides. Environmental factors also play a major role in pesticide degradation. The products of degradation are assimilated by soil microorganisms, and it results in the increased population and activity of microorganisms (Jana *et al*.,1998; Das and Mukherjee, 2000).

Microbial degradation rate depends on certain conditions like soil temperature, soil pH, soil aeration, soil organic matter and frequency of pesticide application (Gavrilescu,2004). Microbial activity is high in warm, moist soils with neutral pH. Physical, chemical and photodegradation have lot of disadvantages like they are highly expensive and their remediation is incomplete which results in the formation of secondary pollutants. Therefore, the effective removal of pesticide from contaminated environment Bio-remediation technique is used.

2.13.1 Degradation and Bio-remediation of Pesticides

The remediation technology must be safe, efficient, economically affordable and should destroy the chemical compound or pollutant without the generation of any intermediates (Frazer, 2000). Currently, several physiochemical methods are inferred for the remediation of contaminated sites and for the treatment of pesticide residues in

the environment. The chemical treatments used are advanced oxidation where it utilizes a powerful transient species, mainly the hydroxyl ion. Percolation filter and adsorption are the basic methods of physical treatments. Along with these, photocatalytic treatment with titanium dioxide (TiO_2) is one of the favored techniques performed for the remediation purpose. Incineration at high temperatures is also being used frequently for pesticide treatment. Alkaline hydrolysis is used under laboratory conditions to remove certain pesticides, but this leads to the formation of secondary pollutants. The physical and chemical remediation methods are,

a) Contaminant- immobilization techniques: - it is a cost-effective in situ approach for the restoration of pesticide-polluted sites. It involves the adsorption mechanisms. Carbonate materials and spent mushrooms are some of the techniques used (Kulshreshtha, 2018; Dad *et al*., 2022).
b) Separation technologies using solvents and synthetic surfactants: - the contaminant is removed from the sludge medium, by using this technology. Solvents, synthetic surfactants, biosurfactants, cyclodextrins, and soil flushing are the different solvent mediums used for the remediation process. The selection of solvent medium depends on the pollutant type to be removed (Ye *et al*.,2014; Mao *et al*.,2015; Odukkathil and Vasudevan, 2016).
c) Fenton advanced oxidation process: - the high utilization of ferrous salts to increase the acidic nature of soil is the base mechanism of the oxidation process. This process is developed to remove organochlorine pesticides (Villa *et al*., 2008).
d) Supercritical fluid extraction of PAH from subcritical contaminated water: - it is the technology used for the recovery of pesticide-contaminated soils. It alters or enhances the solubility of hydrophobic organics by developing water polarity.
e) Electro-kinetic remediation: - it is the detoxification by the use of zero valence iron-nano particles (NVI). This is the potential technique used for Biodegradation of organochlorides (Tummala and Tewari, 2018).
f) Low temperature thermal desorption: - it is an ex-situ cleanup technology to decontaminate pesticide polluted sites at a temperature of 300 and 1000oF. This results in volatilization of the compounds without damaging it. Then the

organic compounds are treated by passing through a burner and convert the gas into liquid phase (Parte *et al.*,2017).

g) Incineration: it causes the complete destruction of the contaminant. The organic compounds are oxidized by applying heat and O_2. The process carries out through two stages. The first stage is the partial oxidation and the volatilization of the organics by heating between 1,000 and 1,800^{o}F. Second stage is the complete destruction of the compound by treating at the temperature below 1,600 and 2,200^{o}F and it is turned into ashes, that can be disposed to a landfill (Parte *et al.*, 2017). In high temperature incineration, pesticides are packaged and then transported to a country with the facilities to dispose hazardous wastes.

The two leading innovative waste disposal mechanisms are composting and waste to energy conversion methods. Aerobic compost, vermicomposting, land filling, incineration, adsorption, pelletization, bio methanation are the examples of waste disposal methods adopted in some parts of India. However, these methods are not cost-effective, highly expensive, and incomplete remediation with the formation of secondary pollutants (Singh and Thakur, 2006). The average estimated cost of these techniques varies between 3,000 to 4,000 USD/ton (Ortiz-Hernandez *et al.*, 2014).

Phytoremediation: - it is cost effective and innovative technology that uses plants to remove the pollutants from soil and water (Raskin and Ensley, 2000; U.S.EPA, 1999). Plants acts as filters and metabolize the substances produced by the nature. Abundant studies have been done to determine effectiveness, similarities, and dissimilarities of plants in the remediation purpose (Hall *et al.*,2011). Phytoremediation of pesticides were studied by researchers. Xia and Ma, (2006) studied the phytodegradation and plant uptake of ethion by water hyacinth. Macrophytes like lemna minor exhibit highest removal efficiency of fungicides, and Chlorpyrifos from water (Dosnon-Olette *et al.*,2009; Prasertsup and Ariyakanon, 2011). The relative growth rate (RGR) and bioconcentration factor (BCF) of plants is positively correlated with the rate of phytoremediation (Riaz *et al.*,2017). Salam *et al.*, (2017) studied the augmentation potency of sugarcane along with candida in the removal of lindane from the doped soil. Atrazine was removed from eutrophicated lakes in China by potamogeton plant and Myriophyllum (Tang *et al.*, 2017). It is also degraded by prairie grass (Khrunyk *et al.*, 2017), and shrub willows (Lafleur *et al.*, 2016). *Acorus calamus* removed

chlorpyrifos under lab conditions (Mahar *et al*., 2016), and Italian grass removed terbuthylazine in liquid conditions (Mimmo *et al*., 2015). Mitton *et al*., (2016) reported the degradation ability of edible crops such as tomato, sunflower, soyabean and alfalfa fo the removal of endosulfan. Nurzhanova *et al*., (2015) studied the elimination capacity of biofuel crop, Miscanthus on the removal of organochlorines. Viktorova *et al.,* (2014) reported the degradation ability of transgenic tobacco plants. Ye *et al*., (2014) developed an ex-situ soil washing technology by the use of maize oil and carboxymethyl-β-cyclodxtrin. Becerra-castro *et al*., (2013) enhanced the degradation efficiency of *Cytisus striatus* by the addition of microbial inoculum. The dissipation rate increased by 2.5 times by the association of inoculum (Rissato *et al*., 2015).

Biodegradation is a natural process whereas, bioremediation is a technology. It is an innovative technology used to treat soils, sediments, sludge, solid-matrix, and ground water without disrupting normal activities (Suthersan, 2001). It is cost effective, safe, convenient and economically feasible and attractive clean-up technology (Klecka,1993; Sheldon *et al*.,1997). In bioremediation the rate of microbial degradation is enhanced by the addition of carbon sources or electron donors and results in the complete mineralization of contaminants to water and carbon dioxide without the buildup of intermediates. American Academy of Microbiology defined bioremediation as the use of living organisms to reduce or eliminate environmental hazards resulting from accumulations of toxic chemicals or other hazardous wastes.

Bioremediation is the most cost- effective, eco-friendly method compared to physical and chemical remediation methods (Saaty & Booth, 1995). They have been used successfully in many countries (Ritmann *et al*.,1988; Galli, 1994). Schrijver *et al*.,(1999) stated that actinomycetes have a considerable possibility for the biotransformation and biodegradation of pesticides. They have been found to degrade pesticides organophosphates. Bio-remediation helps to eliminate, reduce, isolate or stabilize a contaminant or a group of contaminants or various chemical pollutants from environment (Gavrilescu,2005; Liu et al.,1990, Crawford,2004; Singh & Ward, 2004). Bioremediation Bio-remediation is the new bio based developing fields of environmental restoration (Foght *et al*.,2001). The rate of microbial degradation is enhanced by supplementing with nutrients, carbon sources or electron sources (Viggiani et al.,2006). This process is carried out at a quicker rate by microbes or

adding enriched culture of microbes (Singh, 2008). Several research studies show the involvement of microorganism consortia in degradation (Singh and Ward, 2004; Van Hamme, 2004; Dua *et al*., 2002). It partially or completely convert the xenobiotic compounds (Gavrilescu,2005). In majority of studies of bioremediation results in the complete disappearance or mineralization of the hazardous chemicals to water and carbon dioxide without the build-up of intermediates (Frazer,2004). The process is mainly mediated by microorganisms which includes bacteria, fungi,actinomycetes, protozoa etc. (Becaert *et al*.,2001; Wenzel *et al*.,1999). The rate of biodegradation varies a s a function of microbial composition, environmental factors such as pH, temperature, and availability of sunlight (Ragnarsdottir, 2000).

Microorganisms are the efficient bioremediation agents and the fate of pesticides in environemet is affected by microbial activity. A great majority of pesticides are readily degraded by microbes whereas, few of them are recalcitrant (Waldman,1993; Becaert *et al*.,2001, Ward & Singh, 2004). For example pesticides like malathion and parathion appears to be best degraded by microbes. A diverse group of bacteria including members of *Alcaligenes, Flavobacterium, pseudomonas, Rhodococcus* degrade pesticides. For an ideal microbial degradation, it depends not only on the presence of microbes but also on environment parameters (Aislabie *et al*.,1995; Wackett,2001). Requirements for soil bio-remediation by microorganisms includes environmental factors like soil moisture-25-85%, oxygen, redox potential of Eh>50millivolts, nutrients especially nitrogen and phosphorous, pH between 5.5 – 8.5 and optimum temperature between 15-45°C (Chang *et al*.,1996; Di Angelo & Reddy, 2000,). Bio -remediation process is classified into two categories, ex situ and in situ. The ex situ remediation involves technologies like the use of bioreactors, biofilters, landfarming and composting methods. The in situ includes biostimulation, bioventing, biosparging, liquid delivery systems and composting methods (Ward & Singh,2004; Vidali, 2001,Dupont *et al*.,1998, Parte *et al.,*2017).

2.13.2 Biodegradation

Biodegradation is the process by which microbial organisms break down organic materials into smaller molecules with the help of enzymes produced by them. In general, biodegradable materials is organic matter, such as plant and animal matter, other substances derived from living animals or synthetic materials that are

substantially like plant and animal matter to be metabolized by microbes. Some microbes have an incredible naturally occurring, microbial catabolic diversity that allows them to breakdown, change or accumulate a wide variety of substances, including hydrocarbons such as oil, PCB's, PAH,s, pharmaceuticals, pesticides, radionuclides and metals. Although there are many different biodegradation mechanisms, the end product is usually carbon dioxide or methane. Organic material can be broken down either anaerobically (without O_2) or aerobically (with O_2) (Chen *et.al*.,2009).Microorganisms, mainly bacteria and fungi are the two major entities involved in the degradation and transformation of pesticides. Pesticides are used by microorganisms as their carbon and energy sources. Pesticide degrading microbes are isolated from different pesticide contaminated sites such as agricultural lands, sewage sludge, activated sludge, wastewater, sediments, areas around the chemical manufacturing industries, and natural water sources. The intracellular and extracellular enzymes of the microbes play a prominent role in the degradation of the pesticides.

2.13.3 Biosurfactant

The two factors which contribute towards the non-bioavailability of pesticides are low water solubility and high affinity with soil organic matter (Singh V, 2012). The bioavailability of pesticides can be increased by surfactants and thus the pesticide degradation also increases. The use of biosurfactants is a promising tool in bioremediation and waste treatment (Mulligan, 2005; Noordman & Janssen,2002).

2.14 Environmental Fate and Impact of Pesticides

In 1920's public concern about the use of pesticides emerged, because of the presence of pesticide residue in the food products.One of the major sources of Persistent Organic Pollutants (POP'S) in the environment is pesticides. POP'S are poisonous compounds that are released into the environment as a result of a variety of human activities, causing harm to ecosystems, wildlife and people.

Pesticides are often persistent in nature, remain in soil and sediments (Gavrilescu 2004; Maloney 2001). They are ling living, harmful substances released into the environment (Falandysz *et al.,*1994). For example, organochlorine insecticides are found in surface water nearly 20 years after they were first used (Smith *et al*.,1997;

Foght *et al*.,2001). Even decades later, the spot where chemicals are spilled tends to remain the places with the highest concentration of toxins (Buccini, 2004). The persistent nature of pesticides depends on the physiochemical properties of the chemical and pesticides pose a threat to the environment (Basrur, 2002, Bumpus & Aust, 1987). There are several reasons that pesticides persist in environment like nutrient shortage, lack of conditions necessary for their degradation, absence of microbes able to degrade at the contaminated site and the resistant nature of chemicals to biodegradation (Kaufman, 1983; Suthersan, 2004; Huang *et al*.,2000; Gavrilescu, 2006).

Alarming levels of pesticides is reported in air, water, soil as well as in foods and biological materials from India. Out of total pesticides applied only 1% make contact with the target pest, while the remaining 99% drifts into the environment which contaminates both water and biota (Ramakrishnan *et al*.,2015;Pimentel & Fois, 1986; Di *et al*.,1998).Pesticides also leach from soil and contaminates the ground water (Kookana *et al*.,1998) or it persist on the topsoil and became harmful to microorganisms, plants, wildlife and man (Sarkar *et al*.,2009).The accidental spills occurring during the transport of pesticides also pollutes the environment. As a result, intentionally or unintentionally soil and water bodies become the ultimate reservoir of all kinds of pesticides Pesticide degradation in soil is dependent on the physical and chemical properties of the soil pH and microbial community (Bandopadhyay *et al.,*2021).

Modern agriculture techniques demonstrate an increase in the use of pesticides because of the increasing food demand of the rising population which results in environmental damage. The widespread usage and accumulation of pesticide residues is hazardous to all ecosystems (Anand *et al*.,2015). Many factors influence the destiny and movement of pesticides in the environment like interactions with soils, surface water and ground water as well as countless biological, physical and chemical reactions. Other important factors like chemical and physical properties of the pesticide like solubility, soil adsorption, half-life and handling practices of the pesticide user also determines the fate of pesticides in the environment (Gavrilescu, 2005). Pesticides go through several simultaneous processes like emission, wash-off, degradation, sorption or desorption, volatilization, leaching, runoff and plant take during their transformation and movement in the environment (Mackay *et al*.,1997).

Among this degradation is the major process of loss for most pesticides after their application. Pesticide degradation is the breakdown of pesticides within the environment (Chen *et al*.,2005, Singh,2004).

Main three process which helps for pesticide degradation are microbial degradation, chemical degradation and photodegradation. Degradation process change most pesticide residue in the environment into harmless compounds (Leeson *et al*.,2014; EdgeHill & Fin,1983; Whitforis 1995; Foght *et al*.,2001). In rare cases, the products of degradation can be hazardous (Aksu,2005). The chemical degradation occurs by hydrolysis, oxidation-reduction and ionization (Neely,1985; Larson *et al*.,1999). Photodegradation is the breakdown by pesticides by sunlight (Katagi,2004; Remucal,2014; Burrows *et al*.,2002; Li *et al*., 2021). Microbial degradation is the pesticide metabolism by micro-organism, and it is frequently the primary source of degradation in soils (Waldman & Shevah,1993; Edgehill & Fin, 1983; Haque & Freed, 1974).The potency of pesticides differs with respect to changes in environmental conditions of soil moisture, organic amendments, pH and light. The persistence of pesticides in soil and leaching behavior are the two potential risks associated with the application of pesticides. The persistence nature of pesticides is often used as a key indicator for the environmental impacts of pesticide use. The risk group of human beings exposed to occupational poisonings of pesticides is categorized into three clusters. (figure no:-). According to ICMR reports nearly 1 million deaths and chronic illnesses are reported every year due to pesticide poisoning worldwide.

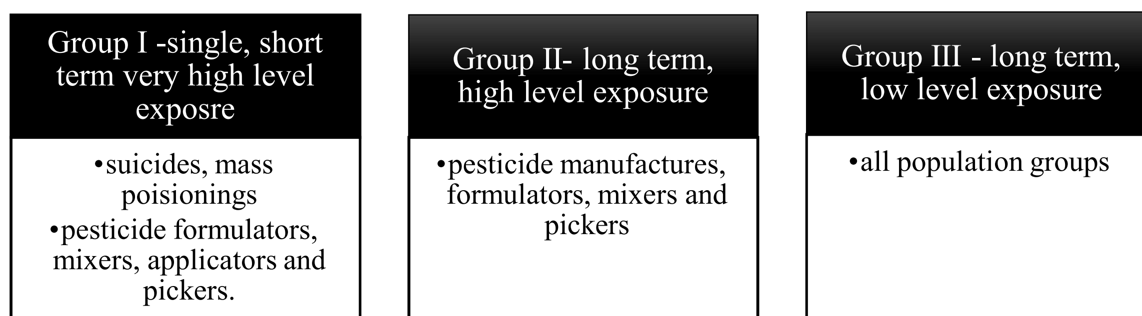


Figure 2.21 Risk groups of occupational exposure (Mes *et al*.,1984).

Pesticides enter the food chain and contaminate natural resources thus posing a huge risk to human health. Chen *et.al*.,(2015) reported that pesticide pollution created the build-up of “cancer villages” and the mortality rate of cancer is higher because of

pesticide use. The endocrine disruptor compounds in pesticides increased the risk of obesity and neurological disorders in migrant workers and their offspring (Perez, 2016).

2.15 Soil Physiochemical parameters and Microbial diversity

Soil is the most complex and vibrant living system of all microbial habitats, which consists of various bacteria, fungi, actinomycetes etc. The soil microbial community plays a major role in the stabilization of soil structure (Sarkar *et al*.,2009). Microbial community is a most reactive component of a terrestrial ecosystem which are constantly tolerating fluctuations and stress in the surrounding conditions than plants and animals. Several environmental factors affect the diversity and composition of soil microbial community. The factors include climatic conditions like high temperature, snow, vegetation – shady trees improve soil biological microbial, soil type, soil properties (Brockett *et al*.,2012), soil charge and structure. Soil texture is an important factor that determines microbial dynamics. Soil clay content have a positive relationship between soil microbial biomass and soil organic matter whereas sandy soils are lower I organic matter and microbial biomass (Franz *et al*.,1997). Clayey soils with high clay content protect soil microbes from predators and dry stress. Microbes can adapt to new and wide range of soil conditions. Microbial communities of different composition function dissimilar. Soil pH is an effective habitat filter and important predictors of microbial composition. Microbial activity and growth is optimum in soils with neutral pH (6-7) and soil temperature between 20-30°C. Microbial metabolism is detected in snow covered soils at a temperature as low as -20°C. At high temperature soil carbon undergoes mineralization and microbial respiration is increased.

The main source of energy rich substrates for microorganisms is dissolved organic carbon and it also affects soil microbial activity and nitrogen dynamics (Wardle, 1992). This carbon source for bacterial species increases with an increase in organic matter, because organic matter increases the retention of nutrients and moisture level. Bacterial survival rate is high in organic soils. Mortality rates of bacteria is higher in subsoil, than topsoil due to low Nitrogen availability . Intermediate moisture level and soil pore size are the good parameters for the stabilization of microbial community. moisture content plays a major role in nitrogen mineralization and microbial

biomass.Diversity of microbial community depends on or varies considerably across geographic distance. Both environmental variables and spatial events influenced the composition and organization of microorganisms. Significant distance effect and environmental factors tends to affect the composition of microorganisms at intermediate scales (10-3000km).

Use of pesticides, compost or manure and the application of genetically modified microbes alter the structure, soil pH of soil microbial communities, whereas soil physio-chemical parameters, plant species and crop rotation act as the key determinants. Therefore, geographical distance, environmental variability and land management practices plays a major role in shaping the different soil communities. Soil is the ultimate sink of pesticides applied in agriculture (Bishnu *et al*.,2009) and it is also the major source for the entry of large number of pesticides into the food chain.

Soil fertility is greatly influenced by the activities of the soil organisms and temperature. The decomposition of soil organic matter increases with an increase in temperature. The life forms in soil adopt either alkaline or acidic nature of the soil for the survival. The exchange capacity of the soil affected by the clay content and organic matter of the soil. The use of fertilizers increases the content of nitrogen and phosphorous in agriculture land. The growth of higher plants is inhibited in grasslands due to the high magnesium and calcium content in the soil. Soil respiration is one of the criteria used for the evaluation of pesticide toxicity studies. Anderson & Domsch, (1985) proposed the microbial metabolic quotient (qCO_2) as a measure for the detection of the effect of xenobiotic compounds on soil microflora. Ananyeva *et al*.,(1997) used the microbial respiration quotient (QR) to assess the effects of various perturbations in soil ecosystems. Nannipieri *et al*.,(2003) established hydrolysis of fluorescein diacetate method for the determination of soil microbial activity. Dissipation rate is one of the important parameters that determines the fate of pesticides in the environment (Sharma *et al*.,2014). The dissipation rate of pesticides is faster in crops than in soils and the dissipation rates varies according to the changes in environmental conditions. These are the literature studies done so far on the degradation of pesticides.

3. MATERIALS AND METHODS

Soil is the most complex and vibrant living system of all microbial habitats which consists of various bacteria, fungi, actinomycetes etc. the soil microbial community plays a major role in the stabilization of soil structure (Sarkar *et al*., 2009). The continuous use of pesticides, compost and application of several physical and chemical pesticide removal techniques alter the soil parameters and structure of soil microbial communities. Biodegradation, particularly microbial degradation, is an efficient technique for the removal of pesticides from the soil.

This chapter deals with the materials and methods used for the analysis of the physiochemical parameters of soil samples and for the isolation of pesticide degrading bacteria. It also includes experimental setup in lab conditions (*in vitro*) for the pesticide degradation studies.

3.1 Study Area and Sample Description

Tea and coffee plantations at the Valparai plateau were chosen as the study site. Valparai is a taluk and mid-elevation hill station in the Coimbatore district of the Sate of Tamil Nadu in India. It is located 3,500 feet (1100 m) above sea level on the Anaimalai Hills region of the Western Ghats and 10° 22’12.00” N latitude and 76° 58;12.00” E longitude. Valparai receives the highest rainfall in the region during the monsoons (around June to September). The major portions of the Valparai plateau are tea and coffee plantations. Valparai shares its boundaries with both the Tamil Nadu and Kerala. It has proximity to Anaimalai Tiger Reserve, Parambikulam Tiger Reserve, Eravikulam National Park, and the Vazhachal forest division. The map of the study area is shown in Figure No: 3.1.

The study sites include different tea and coffee plantations, forest area, and transition areas (site between an estate and a forest region). The study locations were identified based on the history of repetitive pesticide usage. In total, eight different study sites were selected for soil sample collection and denoted as Site 1 to Site 8 (S1, S2, S3, S4, S5, S6, S7, and S8). The study site, S1, is soil sample from forest area (virgin soil taken as the control sample), S2 and S3 are the estate area were natural plant based pesticides are used, S4 is the transition area between a forest and an estate region, and S5 to S8 are the estate areas where synthetic organic pesticides are used. Soil samples were collected every month from 2018 to 2019 to analyse the soil quality, bacterial

population, and pesticide residue in seasonal patterns. The details of the study area are indicated in the Table 3.1

Table No 3.1: Details of the study sites showing the crops cultivated and pesticides applied in the region

Sample/Site code-Nature of pesticide used	Crops in the site	Pesticides used
S1 (Forest area)	NA	Not detected
S2 (Estate area-Natural)	Tea	Neem oil, Eucalyptus oil
S3 (Estate area-Natural)	Coffee, Pepper	Tobacco tea, Neem oil
S4 (Transition area)	NA	Fenpyroximate, Quinalphos
S5 (Estate area – Organic)	Tea	Propargite, Glyphosate, Quinalphos, Spiromesifen
S6 (Estate area – Organic)	Tea	Ethion +Cypermethrin, Deltamethrin, Glyphosate,
S7 (Estate area- Organic)	Tea	Glyphosate, Propargite, Thiamethoxam
S8 (Estate UPASI- Organic)	Tea	Thiamethoxam, Carbendazim, Quinalphos, Fenpyroximate

3.2 Soil Sample Collection

Soil samples from the study sites were collected from depths ranging from 0 to 10 cm using an auger. A composite soil sampling method was used to collect the soil samples from the study sites (Cline, 1944). For a single set of soil samples, around 10 to 15 soil samples from a single study area were taken, pooled, and thoroughly mixed. Soil samples were collected in sterilised polythene bags, transferred to the lab on ice, and then stored. The samples were air dried and sieved through a mesh of a 2mm pore size. They were maintained with a 40% water-holding capacity and kept at room temperature for further experimental study. (Pennock *et al*., 2008; Sarkar *et al*., 2010)

3.3 Procedures adopted for analysis of soil samples.

The physiochemical properties like pH, electrical conductivity, available nitrogen, available phosphorous, available potassium, organic carbon, micronutrients, and soil texture of soil samples were studied. The pesticide residue in the soil samples were

also studied. Table 3.2 shows the procedures used to analyse the physiochemical properties of the soil samples.

Table No 3.2: Methods used for the analysis of soil samples.

SL.NO	PARAMETER ANALYSED	METHOD USED
1.	Organic Carbon	Walkley and Black method (Walkley and Black, 1934)
2.	Available Nitrogen	Alkaline Permanganate Method (Subbiah and Asija, 1956)
3.	Available Phosphorous	Olsen's Method (Olsen and Sommers, 1982)
4.	Electrical conductivity	Conductivity meter (Piper, 1945)
5.	pH	pH Meter (Garrett *et al.*,1999)
6.	Micronutrients	DTPA Method (Lindsay and Norvell, 1978)
7.	Particle size or Soil texture	Hydrometer Method (Gavlak *et al.*, 2005)
8.	Available Potassium	Digital Flame photometer (Karanja *et al.*, 2009)

3.4 Quantification of Soil Bacterial Population

The pour plate method was used for the quantification of bacterial population in soil samples. The bacteria were isolated by plating dilutions of soils (10^{-5} and 10^{-6}) in saline solution (0.9% NaCl) on nutrient agar and incubated at 30 ± 2^0C for 24-48 hours (Sarkar *et al.*, 2009). The developed colonies were counted in plates and the average number of colonies per three plates was determined. The number of total bacteria (CFU- Colony Forming Unit) in dry weight of soil per gram was determined.

Equation (Equ) 1

$$CFU = \frac{\text{Number of colonies X Total dilution factor}}{\text{Volume of the culture plated.}}$$

3.5 Chemicals and Culture Media

The pesticides of analytical grade (>96%), were acquired from the UPASI and also purchased from HiMedia (Table 3.4). The technical grade pesticides were purchased from agricultural chemical dealers and used throughout the experimental studies. All

the other chemicals, bacteriological media, and solvents used in this study were of analytical grade (Himedia, Merck).

Enrichment culture of bacteria, isolation, and degradation experiments were performed in M9-mineral salts media (Sarkar *et al*., 2010). The composition of Mineral Salt Media (MSM) is shown in Table 3.3.

Table No 3.3. Composition of Mineral Salt Media

Chemicals	Quantity in 1 Liter of Distilled water (g/L)
Glucose - $C_6H_{12}O_6$	7gm
Sodium hydrogen phosphate - Na_2HPO_4	6gm
Potassium dihydrogen phosphate - KH_2PO_4	3gm
Sodium chloride - NaCl	0.5gm
Ammonium chloride - NH_4Cl_2	1gm
Magnesium sulphate - $MgSO_4.7H_2O$	0.1 gm
Calcium chloride - $CaCl_2$	0.02 gm
Iron sulphate heptahydrate – $FeSO_4.7H_2O$	0.03gm

Table No. 3.4 Pesticides selected for the study

Sl. No	Pesticide	Pesticide type	Sub-group present	Toxicity (WHO)
1	Quinalphos	Insecticide	Organophosphate	Moderately hazardous
2	Deltamethrin	Insecticide	Pyrethroid	Moderately hazardous
3	Ethion	Insecticide	Organophosphate	Moderately toxic
4	Propargite	Acaricide	Sufite ester	Slightly hazardous
5	Glyphosate	Herbicide	Organophosphate	Least toxic
6	Fenpyroximate	Acaricide	Pyrazolium	Moderately hazardous
7	Spiromesifen	Insecticide	Tetronic acid derivative	Least toxic
8	Thiamethoxam	Insecticide	Neonicotinoid	Moderetly hazardous

3.6 Selective Enrichment, Isolation, and Maintenance of Bacterial Isolates

The enrichment culture technique was used for the isolation of pesticide-degrading bacteria. The isolation of pure cultures of beneficial microorganisms from natural resources has been based on enrichment culture techniques. This method completely depends on the selection advantage acquired by an organism to utilize a particular chemical compound as carbon or energy source in a medium, containing only other organic ingredients. Repeated transfers of bacteria through enrichment culture increases and improves the degree of selectivity of microorganisms before plating. All the enrichment culture media were autoclaved, and experiments were carried out in airtight flasks shaken at 120rpm, at 30±2°C, and at pH 7±0.2.

A 20 gram of tea plantation soil was transferred to a 50ml sterile beaker and treated with 2mg of respective pesticides dissolved in 100µl of suitable organic solvents along with distilled water and mixed thoroughly (Cycon *et al*.,2014). This treated soil sample was maintained at room temperature for five weeks. The periodic mixing and maintenance of water holding capacity were checked regularly. After pesticide application, 10 grams of tea plantation soil from the beaker was taken and added to 250ml flasks containing 100ml of MSM supplemented with 50mg/L of selected pesticides. MSM samples with pesticides were incubated for three days or seventy-two hours on a rotary shaker (120rpm) maintained at 30±2°C. Samples of 1ml of soil suspension were transferred into flasks containing the fresh MSM supplemented with 100mg/L pesticides and incubated for additional 72 hours under the same temperature and pH conditions. This was followed by seven subsequent transfers into the fresh MSM with pesticides. Serial dilution (10-fold dilutions) of flask samples was done and 0.1ml of the sample was plated onto MSM agar plates supplemented with 100mg/L of respective pesticides and incubated at room temperature for isolation of single colonies. The single colonies were picked up after incubation. Pure cultures were obtained by repeated subculturing. The strains were stored on MSM media at 4°C for further experimental studies.

3.6.1 Preparation of pesticide stock solution

The pesticides selected for this study were Deltamethrin, Quinalphos, Thiamethoxam, Spiromesifen, Fenpyroximate, Glyphosate, Propargite, and Ethionas featured in Table

No 3.5. A stock solution of all the selected pesticides with a concentration of 1mg/ml ie1000ppm was prepared and further working concentration was extracted from the stock solution (Heong *et al*., 2015). The stock solution was prepared by diluting pesticides in their respective organic solvents with high solubility and distilled water. These were stored in glass bottles and kept away from light.

Table No 3.5: Details of the pesticides and their respective organic solvents

Pesticide	Formulation	Trade name	Purity (%)	The organic solvent used for stock preparation
Quinalphos	Liquid	Quinguard	25% EC	Hexane
Glyphosate	Liquid	Glytaf	41% SL	Water
Ethion	Liquid	Nagata	40%	Acetone
Thiamethoxam	powder	Actara	25%WG	Acetone
Deltamethrin	Liquid	Shastra	2.5% FS	Acetone
Propargite	Liquid	Omite	57% EC	Acetone
Spiromesifen	Liquid	Oberon	22.9% SC	Xylene
Fenpyroximate	Liquid	Mitigate	5% EC	Xylene

EC-Emulsifiable Concentrate, SL- Soluble Liquid, WG-Water dispersible granule, FS-Flowable Concentrates, SC-Suspension Concentrate.

3.6.2 Disc Diffusion Assay

The tolerance or survivability of the microbes in the presence of pesticides and the toxicity measurement of pesticides was indirectly carried out by disc diffusion assay (Sharif and Mollick, 2013). For each of the eight pesticides used in the study independently, the resistance of tea plantation soil isolates was examined separately. Each plane disc contained five different concentrations of pesticides 100 ppm to 300 ppm with a 50-ppm treatment interval. For each pesticide under investigation, the media was inoculated with each soil bacterial culture separately, and the disc without pesticide stress (0 ppm) was regarded as the test control (culture with optimal growth). As a result, each bacterial isolate received a total of 5 pesticide treatments, along with a control. The agar media is inoculated with respective bacterial strains and the discs were placed in the agar plates. After 24 hours, the zone of inhibition was calculated.

3.7 Characterization of Tea Plantation soil bacterial isolates

3.7.1 Morphological characterization

Pure culture of pesticides degrading bacterial strain was isolated and grown on nutrient agar plates and incubated overnight at 30°C±2 for 24 to 48 hours. The following methods were employed to study different aspects of bacterial morphology.

3.7.1. Colony morphology

Single pin head colonies of isolated bacterial strains were selected and visually observed. The morphological features such as colony colour, colony form, colony elevation, colony margin, colony surface, and pigmentation of the isolate were recorded (Breakwell *et al*., 2007). Bergey's Manual of Systematic Bacteriology was also used for identification purposes (Bergey, 1994).

3.7.1.2. Gram staining

The Gram staining test, a standard protocol (Bartholomew and Mittwer, 1952), was applied to determine the morphological characterisation of the bacteria. A single colony was selected from the fresh overnight-grown culture of bacterial isolates and placed on a sterile glass slide. The culture on the glass slide was mixed by adding a drop of sterile distilled water with the help of a sterilized loop to obtain a uniform bacterial smear. The bacterial smear was heat fixed and stained with primary stain crystal violet for 1 minute followed by water and alcohol wash (70%) for 20-30 seconds. Final staining was done with safranin for 1 minute. The slides were washed and kept for drying. The stained bacterial smears were observed under an oil emersion lens (100x) through a light microscope and the cell characteristics were noted.

3.7.2 Biochemical Characterization

Biochemical characterization of native lab isolates was conducted taking into consideration tests for identification of prokaryotes after the initial characterization of isolates by morphological tests to determine gram nature, colony morphology, and fluorescence tests. The biochemical tests conducted with native isolates include the MacConkey agar test, urease test, citrate agar test, catalase test, and oxidase test.

Lactose fermentation test was done using MacConkey agar media. The bacterial isolates were streaked on the prepared MacConkey agar plates. The colour change of

media from pink to yellow was marked as positive result. Urease test was performed by inoculating the isolates in Christensen's Urea Agar. The positive result is marked by pink colour when kept for a week. The citrate utilization test was performed by inoculating the isolates in Simmons citrate agar containing bromothymol blue indicator. After 24 hours of incubation at 37° C, a change in colour of the culture medium from green to blue is noted. (Cappuccino and Sherman, 2002). Drops of H_2O_2 was placed on a sterile slide, for the catalase test, and then the bacterial isolates were aseptically seeded. The appearance of air or gas bubbles indicated positive results. For the oxidase test, bacterial isolates were applied to the surface of the oxidase discs that resulted in purple colour on the discs after 5 seconds, marked as positive result (Prescott *et al*., 2005).

3.7.2.1 Antibiotic Susceptibility Test

Pesticides degrading isolates (selected through enrichment culture) were tested for sensitivity towards different antibiotics. The antibiotics selected for the test were Ampicillin(AMP), Carbenicillin(CAR), Chloramphenicol(CMP), Kanamycin(KAN), Penicillin-G(PEN), Polymyxin(POL), Spectinomycin(SPC), and Tetracycline(TET). Antibiotic discs were procured from Himedia as sets of different individual antibiotics (at 25/30/100 mcg concentration per disc) given for bacterial isolates. The bacterial isolates were subcultured freshly in nutrient broth and incubated at 30°C. The overnight grown fresh culture (0.1ml) of all the isolated strains was inoculated individually on the Mueller Hinton Agar (MHA) plates by spread plate method under aseptic conditions, and different antibiotic discs were placed at appropriate distances on agar plates using sterile forceps. The inoculated agar plates were then incubated for 24-48 hours at 30°C and observed for the appearance and non-appearance of the zone of inhibition around the antibiotic discs. The appearance of the zone of inhibition indicates that the bacterial strain is sensitive to the antibiotics, whereas, the non-appearance of the zone of inhibition indicates the resistance of the bacterial strain to the antibiotics (Sundari *et al*., 2019). The standard chart for zone diameter interpretation is given in the Appendix section II.

3.7.3 Molecular Characterization

3.7.3.1 DNA isolation

DNA was extracted from a loopful of well-grown bacteria following the standard phenolchloroform method (Sambrook and Russel, 2006

000). Briefly, the culture was well resuspended in Lysis buffer (NaCl 400 mM, Sucrose 750 mM, EDTA 20 mM and TrisHCl 50 mM), containing 1 mg ml^{-1} lysozyme and incubated at 37 °C for 1 hr. Subsequently, SDS (1%) and proteinase K (100 µg ml^{-1}) were added to the solution and the incubation was continued for 2 hrs at 55 °C. The crude DNA sample was extracted with chloroform: isoamyl alcohol (700 µl of 24:1 mixture) twice and the aqueous phase containing DNA was retrieved by centrifugation at 10000 rpm for 10 min. Further, 0.6 volume of isopropanol was added, and the DNA was allowed to precipitate at −20 °C for 60 min. The DNA was pelleted and then washed times with 70% ethanol, air dried at room temperature for 20-30 min and dissolved in TE buffer (~30 µl).The quality of DNA was confirmed by agarose gel electrophoresis. The primers used were Universal primers 27F (Forward Primer): AGAGTTTGATC(AC)TGGCTCAG and 149R (Reverse primer): GGTTACCTTGTTACGACTT.

3.7.3.2 PCR (Polymerase Chain Reaction)

The 16S rRNA genes of bacterial DNA were amplified using the PCR technique in a 20 µl reaction volume containing 1µl DNA (10–50 ng), 1µl each of Forward and Reverse primers (10 picomoles µl-1), and 10µl Emerald Amp GT PCR master mix (Takara).

3.7.3.3 The Cycling Conditions

The initial denaturation was done at 95 ◦C for 2 min, followed by cycle denaturation at 95 ◦C for 40 s, annealing at 55 ◦C for 40 s, extension at 72 ◦C for 1.5 min for a total of 30 cycles, and a final extension for 10 min at 72 ◦C. The success of the PCR reaction was confirmed by running 5µl PCR product on 1 % agarose gel (impregnated with ethidium bromide) at 120 V, for ~45min in 1X TAE Buffer. 100bp DNA ladder from Thermo was loaded parallel to PCR products as a size marker. The PCR products were further treated with ExoSAP-IT product clean-up reagent and were used as a template for sequencing PCR. Sanger sequencing PCR was done with ABI PRISM Big Dye terminator ready reaction mix (Life Technologies, USA) using internal primer 1090 R/357 F and ABI3730xl DNA analyzer (Applied Biosystems). The sequences were quality-checked and trimmed using the Software sequencher

V4.10.1 (Gene Codes Corporation, Ann Arbor, MI USA). Trimmed sequences were searched in NCBI using the BLASTn tool and the identity of the sample was confirmed based on percentage similarity and query coverage of the nearest neighbors.

3.7.4 Phylogenetic tree construction

For phylogenetic analysis, the 16S rRNA gene sequence of the isolates were aligned with sequences from the database using CLUSTAL-W multiple sequence alignment tool. MEGA X version 11 was used to conduct phylogenetic analysis using the neighbour joining method.

3.8 Growth of bacterial isolates in different carbon and nitrogen sources

The bacterial strains were grown in different carbon sources and nitrogen sources, and in respective pesticides for 10 days for the analysis of bacterial preference towards different energy sources. Inoculum size 0.5 OD To 250ml of MSM 1.75gms of carbon sources (Glucose, Galactose, Sucrose, and Maltose), nitrogen sources (Ammonium nitrate, Ammonium chloride, Ammonium sulphate, and Urea), and respective pesticides were added, and the OD was recorded at 600nm. The MSM with bacterial culture without any kind of energy sources was kept as the control (Jiang *et al*., 2019)

3.9 Degradation Studies

3.9.1 Preparation of bacterial inoculum for degradation studies

A single colony of a pure bacterial culture was collected from the related bacterial plates under aseptic conditions and introduced into separate test tubes filled with 10 ml of sterile nutrient broth. Bacterial isolates were grown overnight in nutrient broth at 30°C±2 and 150rpm for 48 hours. The bacterial culture was centrifuged at 6000rpm for 20 minutes at 4°C for the cell pellets. The bacterial pellets were then washed twice with 0.85% saline solution and suspended in 10 ml of sterile water. The bacterial cells in sterile water were used as inoculum for degradation studies (Sundari *et al*., 2019). All the soil bacterial isolates exhibited considerable growth and can subsequently be served as bacterial inoculum for all assays (Jaikaew *et al*.,2017). The degradation kinetics and half-life of the pesticide were calculated using equations (2) and (3).

Equu (2) ……….$C_t/C_o = e^{-kt}$

C_o = amount of pesticide at time zero, C_t=amount of pesticide at time 't', k & t = rate constant

t = days

Equ (3) ………..$t_{1/2} = C_o/2K$

$t_{1/2}$ = Half-life.

3.9.2 Optimization of different parameters for degradation in liquid MSM

To determine optimal conditions of bacterial growth and pesticide degradation, the effect of pH, temperature, and bacterial inoculum size variations were studied (Wu *et al*., 2021)

3.9.2.1 Optimization of Temperature

Temperature with a range of 15°C to 40°C was with an interval of 5°C unit variation (based on meteorological data of the study site) selected for the optimization studies. When one temperature is selected, the pH 7 and inoculum size (OD @ 0.5) were kept constant. 100µl of overnight grown cultures was added aseptically to 10ml broth of varying temperatures, with 100ppm of respective pesticides and incubated in a shaking incubator at 150rpm and the OD was recorded.

3.9.2.2 Optimization of pH

pH with a range of pH 5 to pH 9 with an interval of one pH unit variation was studied. The pH was adjusted to different values by using standard HCl and NaOH solutions. 100µl of overnight grown cultures was added aseptically to 10ml broth of varying pH (pH 5 – pH 9) with 100ppm of respective pesticides and incubated at 30±2°C in a shaking incubator at 150rpm. The OD was recorded.

3.9.2.3 Optimization of bacterial inoculum size

Bacterial inoculum sizes with a range of 0.5 to 2.5 (OD @600nm, 0.5 OD ≈ 1.5 x 10^8 cells) were selected for the optimization studies. When one particular inoculum size is selected, the pH(7) and the temperature(30±2°C) are kept constant. 100µl of overnight grown cultures was added aseptically to 10ml broth of varying inoculum size with a

pesticide concentration of 100ppm and incubated in a shaking incubator at 150rpm and the OD was recorded.

3.9.3 Optimization of the parameters by Taguchi OA Methodology

Three parameters were chosen were chosen based on preliminary single factor studies.The schematic representation of the various steps involved in the optimization study by Taguchi OA (Roy, 2001) methodology is shown in three factors and their three levels which had a considerable impact on the microbial growth and pesticide degradation. In this step, the Taguchi DOE methodology was used to design the matrix based on the appropriate selection of OA, which in turn was based on the number of operational process parameters and their levels. In this study, L-9 OA was selected, and biodegradation experiments were performed. The Minitab software was used to design the OAs and to analyse the experimental data to determine the level of influence of individual factors, the mutual interaction between selected factors, the establishment of the optimum process conditions, and the evaluation of the process performance. Signal to-Noise (S/N) ratio with the 'larger is better' feature was chosen as the indicator of the performance quality to analyse the process response (microbial growth and pesticide degradation) under the current as well as optimum conditions (Rasoulifard *et al*., 2015). The S/N ratio can be defined as the quality attributes deviating from the targeted value of the output. The S/N values of the experimental responses can be calculated from the following equation (4)

$$SN_L = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_i^2}\right)$$

Where y is the average of the output (i.e., biomass growth and pesticide degradation percent), and n the number of replications of each trial. Validation of the result was done by conducting the confirmatory biodegradation experiment under the optimized process parameters predicted by Taguchi Methodology.

3.9.4 Esterase Assay

Each bacterial isolate was utilized separately in the assay in combination with cell-free extract (CFE) serving as a crude enzyme representation. A reaction tube containing 100 L of CFE, 4.8 ml of 40 mM phosphate buffer (pH 6.8), and 100L of 0.3 mM -alpha napthyl acetate as an enzyme substrate was added. The assay mixture

was incubated at room temperature for 20 mins in the dark. After incubation, the mixture was stained with 1ml of a solution containing 1% fast blue B salt in a 40mM phosphate buffer and 5% SDS. For colour development, tubes holding the reaction mixture underwent one final incubation at 20°C for 30 min. At 590 nm, a change in the colour of the reaction mixture indicative of the esterase activity of the isolate was seen. From the standard plot of -naphthol, the amount of substrate (- alpha napthyl acetate) converted to product (-naphthol) was estimated and expressed as U/min/ml (Sundari *et al*., 2019).

3.9.5 Degradation Experiments in Soil

To investigate the effectiveness of the pesticide-degrading strain in the bioremediation of pesticide-contaminated soils, experiments using tea plantation soil were performed. Autoclaved sterile soil samples were used for the study. Five hundred grams (500gms) of each type of soil was placed in a 5 L Erlenmeyer flask, and the moisture content was adjusted to 40%. The soil moisture content was maintained at a constant level throughout the experiment by the addition of distilled water when necessary. Pesticides were added to a final concentration of 100 mg/kg. After mixing, a suspension of the respective bacterial culture to inoculate the soil (in triplicate) at a final concentration of 1.0×10^6 CFU/g was added. After inoculation, the soil was incubated at 30°C. Soil treated with the same amount of pesticides without bacteria served as uninoculated control samples. Twenty grams of soil were collected from each sample on days 15, 30, 45, 60, 75, and 90 for analysis of the residual pesticide concentration by GC-MS (Cycon *et al*., 2014). The degradation percentage was calculated using equation depicted below:

Equ (5)

$$\text{Degradation (\%)} = \frac{\text{Initial concentration of pesticide (ppm)} - \text{Final concentration of pesticide (ppm)}}{\text{Initial concentration of pesticide (ppm)}} \times 100$$

3.10. Preparation of microbial consortium for the degradation of the pesticide Deltamethrin

Microbial consortiums were prepared from the isolated bacterial strains to degrade the pesticide deltamethrin.

3.10.1 Co-habitation plate Assay

Consortia formulation and associated studies can be conducted after confirming the occurrence of any antagonistic effect among the native isolates in the study. All ten native bacterial isolates were tested in different combinations for antagonism in vitro cohabitation studies conducted on NA media plates. Fresh inoculum of all the isolates was prepared, and tea plantation soil bacterial cultures were grown overnight on Luria agar at 30°C and 180 rpm in an incubator shaker. A single pure colony of bacterial isolates was picked using a sterilized toothpick and inoculated following the method of pairwise co-culturing where one isolate was streaked on one half and the other isolates on the other half of 9 cm NA Petri plates. Similarly, triplet and quadruplet combinations were also made. Nutrient agar plates were inoculated accordingly at 30±2°C for 48 hrs. After, post-incubation, plates were checked for formations of zones of inhibition indicative of antagonism amongst the bacterial strains at the point of convergence of two counterparts. The co-culturing experiments were carried out in triplicates and repeated twice (Gilbert *et al.*, 2003; Sundari *et al.*, 2019).

3.10.2 Degradation of Deltamethrin by Bacterial Consortium

To assemble a bacterial consortium all different possible combinations of the selected bacterial strains were made. The microbial consortia were formulated by mixing equal proportions of 1 OD (at 600nm) pure suspension of each selected bacterial isolate. MSM in flasks supplemented with deltamethrin (100 ppm) was inoculated with these consortia (1 % v/v). Triplicate flasks were kept for each consortium and un-inoculated flasks were kept as control. They were incubated at room temperature (30 ±2°C) in a rotary shaker at 120rpm. Aliquots from each flask were serially diluted and spread-plated at regular intervals to confirm the growth of every member of the consortium (Sasikala *et al.*, 2012; Varghese *et al.*, 2021). The degradation analysis was done using GC-MS to select the best consortia.

3.11 Analytical technique to study pesticide degradation

The pesticide residue analysis and the metabolites produced by the degradation of pesticides in liquid MSM were detected by GC-MS analysis. The pesticide residue analysis was done by the USEPA 3540C protocol. This is a procedure used for the extraction of non-volatile and semi volatile organic compounds from solids such as

soils, sludges, and wastes. This method is applicable to the isolation and concentration of water insoluble and slightly water-soluble organics in preparation for a variety of chromatographic procedures. Soil samples were extracted using either acetone/hexane (1:1) (v/v) or by using methylene chloride/acetone (1:1) (v/v) (Abraham *et al.*, 2014).

For the pesticide concentration determination through GC-MS analysis 5ml of liquid MSM samples or 10 gm of soil were taken and filled to volume of 20ml with deionised water. It was extracted twice with 10 ml of ethyl acetate on a rotary shaker (30 minutes). After that, the sample was centrifuged for 5 minutes at 8000g. The supernatant after centrifugation was filtered and dehydrated with anhydrous Na_2SO_4. Then the sample is extracted with acetonitrile or dichloromethane or respective solvents and vortexed for 2mins. After that the obtained sample was dried using a rotary evaporator. The dried sample was extracted using hexane or acetone, and the volume was reconstituted about 15ml and used for GC-MS analysis to determine the concentration of pesticides in the samples. The solvents used for extraction varies depending on the pesticides needed to be determined. The solvents used were dichloromethane/ acetonitrile/ ethyl acetate (Rana *et al.*, 2015; Hegde *et al.*, 2017). The properties of GCMS Program is depicted in the Appendix section of III.

3.12 Biochemical analysis to identify biomolecules for biodegradation

Biochemical experiments were carried out to determine the qualitative existence of biomolecules regarded as potentially suitable for biodegradation by bacteria. All the native soil bacterial isolates were used separately for each of the biochemical tests and each test was double verified. The analysis done was biosurfactant production and biofilm formation.

3.12.1 Biosurfactant Production

The production of biosurfactant rhamnolipid by all the bacterial isolates was investigated independently using the procedure adopted from Verma *et al.*, (2006). Agar was prepared and autoclaved, then supplemented with 0.2 g CTAB and 0.005 g methylene blue to give the media a light blue colour. Overnight developed bacterial cultures were spot injected on the agar plate three times with a consistent distance between each spot. As a control, an uninoculated media plate was used. The plates were incubated at 30°C for 5 days. They were checked every day for the presence of

dark blue pigmentation around the bacterial colonies, which can be reported as a positive test for biosurfactant production.

3.12.2 Qualitative Detection of Biofilm

3.12.2.1 Tube Method

The tube method (TM) also known as the Christensen method is an assay done for the qualitative detection of biofilm-producing microorganisms, by observing the occurrence of visible film. The selected bacterial isolates were inoculated in polystyrene test tubes with a 3ml volume of Tryptic Soy Broth (TSB) and inoculated for 48 hours at 37°C (Christensen *et al*., 1995). After the inoculation, the test tubes are washed twice with buffer solution and stained with safranin solution and placed for drying. The assay was considered positive when a visible film could be seen lining the test tube wall and bottom. The observation of the stained ring at the air-liquid interface was considered negative.

3.12.2.2 Congo Red Agar Media

The bacterial isolates were inoculated in the Brain Heart infusion Agar (BHA) with congo red agar stain and kept for 48 hours overnight at 30°C ± 2, in the incubator. The colour of the colonies was observed. The dark black coloured colonies are considered positive for biofilm formation. The colourless colonies were considered as negative for biofilm formation (Cotter *et al*., 2009).

3.13 Statistical Analysis

The results of the triple analysis were expressed as the mean standard deviation. A $P < 0.05$ value was considered statistically significant. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS) and included one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). Pearson's correlation coefficient (Microsoft Excel program) was used to analyse soil properties. The Kolmogrov Smirnov test was used to determine the normality of the pesticide residue data, and it did not meet the normality assumptions. As a result, a non-parametric test, Kruskal Walli's ANOVA, was used to compare seasons. The link between pesticide residue and other indicators was investigated using Spearman's rank correlation.

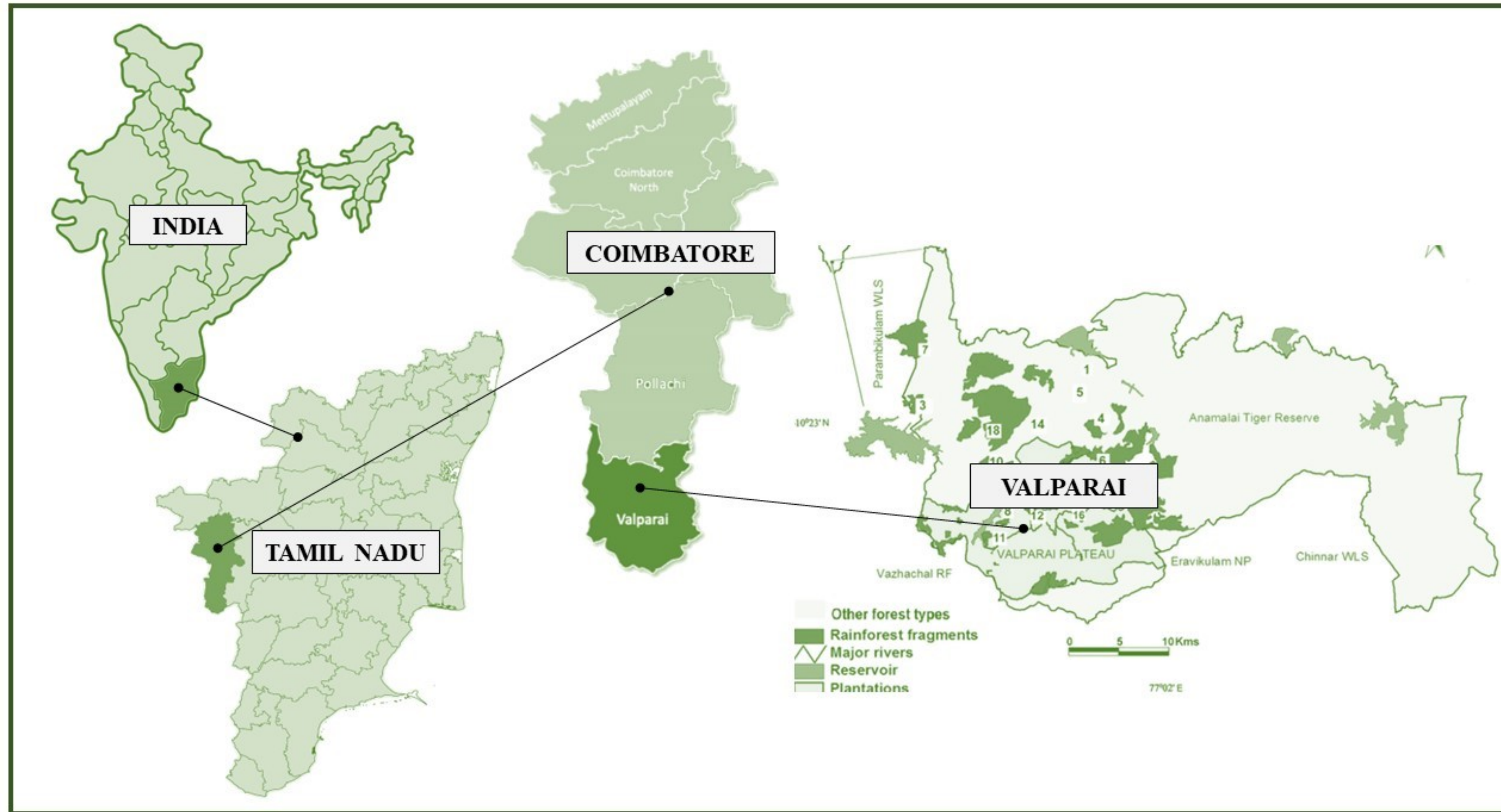


Figure 3.1 Map of the study area showing the tea plantations at Valparai plateau (The number 1,2,3,7,15,14,16,11 denotes the sampling sites) Sidhu *et al*., 2015.

4. RESULTS AND DISCUSSION

The selection of resistant and competent microbial strains is one of the most important steps in successful biodegradation for they play a vital part in the breakdown of pesticides. The indigenous microbes from the contaminated sites provide a dual benefit of detoxifying pollution and they do not endanger other local species of flora and fauna. In the present context, an economically efficient and eco-friendly technique was used for degrading environmental toxins. Therefore, the study focused on the isolation of native microbes from the tea garden soils of the site chosen for the study that possess the ability to degrade the pesticides. The investigation was carried out in a sequential way beginning with the isolation of bacteria, followed by characterization, examination of certain degradation features of the microbes in the preliminary level, and measurement of the actual reduction in pesticide concentration by the isolated bacteria. The findings of the study are explained and discussed in this chapter.

4.1 Soil Physiochemical Parameters

The purpose of soil analysis was to examine the physiochemical properties of soil samples from tea plantation fields in Anaimalai Hills, Tamil Nadu, South India. Soil samples were collected from the site once a month from March 2018 to February 2019, and physicochemical properties were examined using established procedures represented in Table No: 3.3(Kudesia, 2000). The physiochemical parameters such as pH, electrical conductivity, moisture content, organic carbon, macronutrients (N, P, K), and micronutrients (Copper, Iron, Zinc and Manganese) were examined. The sampling period has been distributed into three seasons: pre-monsoon (March–May), monsoon (June–September), and post-monsoon (October–February) based on the metrological data of Valparai (Table No 4.1). The physicochemical parameters were examined in triplicate across all soil samples. The obtained experimental work results are explained in this section.

Table 4.1: Meteorological data of Valparai Plateau, region of Anamalai Hills (2018-2019).

Month	**Temperature(^{o}C)**		**Actual rainfall(mm)**	**Humidity (%)**	**Average sunny days**
	Max	Min			
Pre-monsoon					
March	26	16	150	64	9.0
April	25.4	17.5	343	80	7.3
May	23.9	17.9	382	89	5.0

Month	Temperature(^oC)		Actual rainfall(mm)	Humidity (%)	Average sunny days
Monsoon					
June	21.5	17.2	519	92	3.5
July	20.9	16.7	615	93	3.4
August	21.2	16.6	501	92	3.6
September	22	16.4	350	90	4.6
Post-monsoon					
October	22.3	16.3	393	89	5.3
November	22.3	15.7	243	83	6.2
December	22.7	14.6	85	76	7.2
January	24	13.8	30	66	8.4
February	25.5	16	50	59	9.2

4.1.1 Soil pH

The pH of the solution is defined as the negative logarithm of the hydrogen ion activity. It is determined using an electrometric approach (pH meter) that determines soil acidity and alkalinity. The pH of pure water at 250 degrees Celsius is 7.0; as pH declines from 7.0, H^- concentration surpasses OH^- concentration, and the range becomes acidic. When OHion exceeds Hion, the pH ranges between 7 and 14, indicating that the pH is alkaline.

The pH values in soil samples obtained at different months during three seasons for one year were recorded. The pH of the samples evaluated ranged from 4.23±0.14 to 6.63±0.26 for one year. The value varied in the range of 4.23 to 5.66 during pre-monsoon, 4.808 to 6.35 during monsoon, and 5.63 to 6.63 during post-monsoon. In site S1 (forest area- virgin soil) the pH was in the range of 4.23±0.14 during pre-monsoon, 4.81±0.14 at monsoon, and 5.63±0.35 during post-monsoon. The value during pre-monsoon in S2 and S3 varied at a range of 6.85 ± 0.20 to 5.67 ± 0.09. During monsoon, it was in the range of 6.06±0.09 to 6.36 ± 0.17. in site S2 the pH during monsoon was 5.81 ± 0.20 which shows a slight decrease from pH values of monsoon and pre-monsoon seasons. The pH of S4, a transition region, was 4.39±0.16 at pre-monsoon, 5.27±0.34 at monsoon, and 5.61 ± 0.13 during post-monsoon. The sites S5 to S8 are the estate regions where synthetic pesticides have been applied. The pH of S5 to S8 ranged from 4.78 ± 0.10 to 5.71± 0.12 during pre-monsoon, 5.08±0.09 to 6.08 ± 0.24 during monsoon, and 5.25 ± 0.14 to 6.30 ± 0.17 during post-monsoon.

In all the test sites, the pH of the soil marginally increases during the post-monsoon and slightly falls during the pre-monsoon and monsoon.

Brunner and Sperisen, (2013) categorised soil into strongly acidic soils ($pH<4.5$), and moderately acidic soils (pH 4.6 – 5.5) based on pH value. As a result, the soil samples of the present study can be classified as moderately acidic soils. The pH of soil samples from forest area (S1) and other sites (S4 to S8) were acidic in nature at all seasons. In forest ecosystems, the soils are acidic in nature and the pH also influences the transformation of organic matter in the soil (Tonon *et al*., 2010).

During monsoon, the pH shows a slight decrease in all the study sites. This decrease in soil pH during monsoon is attributable to rainfall leaching of basic cations from the soil. Rousk et al., (2010) observed that rainfall leaching alters the level of basic cations in soil, and this slowly increases the acidic cations, Al^{3+} and H, in the soil. The soil pH is low during pre-monsoon in all the study sites because of the presence of more soluble salts. A similar study done by Jia et al., (2021) reported that soil pH was (9.28) higher in the dry season than that in the wet season (9.11), due to the leaching of more soluble substances due to rainfall. The pH slightly increases during the post monsoon season in comparison with the pre-monsoon season. The application of fertilizers, high moisture content, and rainwater accumulation will increase soil pH during post-monsoon season. Yadav et al., (2017) recorded an increase of pH from 7 to 8.75 during post-monsoon in soil samples from Pune. Kaur et al., (2021) reported that soil pH slightly increased from 7.28 to 7.31 in post-monsoon in their research work. Given the above findings, similar inferences may be drawn in this study. The pH of the soil samples of sites S5 to S8 varies in the range of 4.66 ± 0.2 to 6.5 ± 0.01 during three seasons with the post-monsoon values being near to neutral pH. In general, tea and coffee plants prefer acidic soils for their growth with a pH range of 4.5 to 6 (Bordoloi et al., 2021), and within this pH range most suitable value is 5.13 to 5.33 (Ye et al., 2022). When compared to the sites S1,S2,S3, and S4, the sites S5 from S6 are more acidic in nature. This is attributable to the application of chemical fertilizers and synthetic pesticides in these sites. The use of synthetic fertilizers and pesticides decreases the pH of the soil and increases its acidic nature. The high acidification of soil affects the yield and quality of tea. It also affects the soil microbial community and soil enzyme activities (Prajna et al., 2022). Aikpokpodion et al., (2010) and Ololade et al., (2010) also reported that the application of pesticides

enhances the acidic nature of the soil. Bordoloi et al., (2021) studied the physiochemical properties of tea garden soils of Assam and reported that the prolonged use of pesticides increased the acidification of soil samples.

Even though acidic soils are ideal for the growth of tea and coffee, it is necessary to maintain the soil pH at the optimum level. It also helps to conserve the soil microbial community and soil enzyme activities. The soil pH values of the present study are represented in Figure No: 4.1.

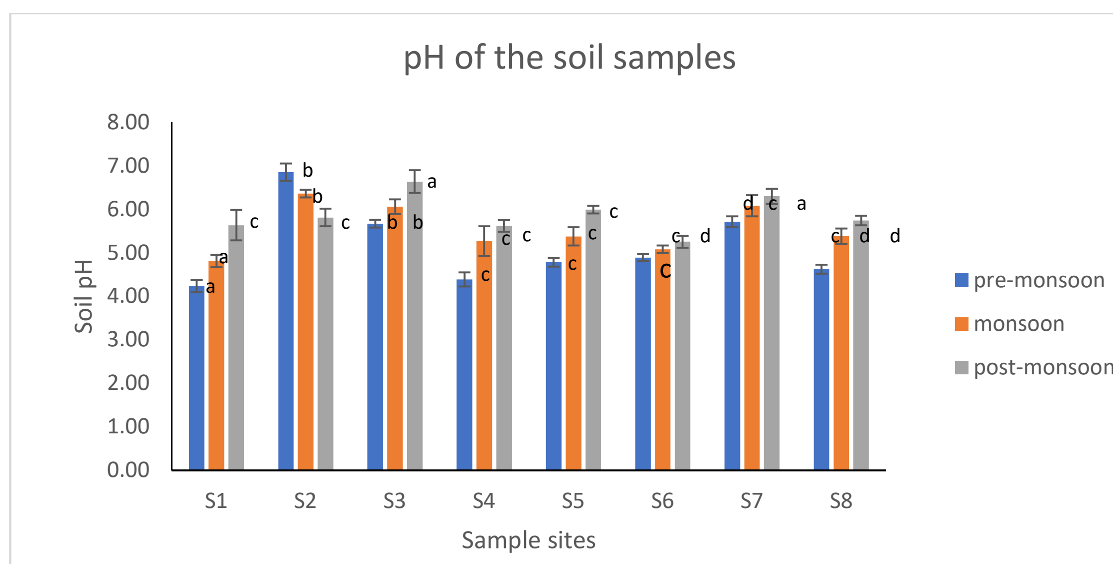


Figure 4.1: Seasonal variation of Soil pH in eight study sites. Values are expressed in Mean±SD. P value (≤0.05). Means that do not share a letter are significantly different.

4.1.2 Moisture Content in Soil

Moisture is a crucial physical feature of soil. The moisture content of soil influences nutrient absorption. The potency and consistency of soil are virtually always dependent on its moisture content. Soil moisture content alters the substrate solubilization and microbial motility. Hence, it is necessary to determine the moisture content of the soil. The moisture content of the soil is demonstrated to vary with soil type, climate, and humus concentration (Noble and Alexander, 1977). It is determined by the void ratio, particle size, clay minerals, organic matter, and groundwater quality. The moisture content of the soil was analyzed in this study using the oven-drying method.

The moisture content of site S1 was 50.49 ± 1.45% during pre-monsoon, 66.18 ± 1.24% during monsoon, and 59.01 ± 0.97% at post-monsoon. The moisture content of

sites S2 and S3 varies at the range of 47.34 ± 1.89% to 47.74 ± 1.85% during pre-monsoon, 60.68± 1.75% to 64.25 ± 2.82% during monsoon, and 56.68 ± 0.89% to 58.89 ± 5.39% during post-monsoon. Site S4 (transition area) shows moisture content in the range of 30.80 ± 0.77% during pre-monsoon, 48.13 ± 1.87% during monsoon, and 38.13 ± 1.84% during post-monsoon. In sites S5 to S8 the moisture content varies in the range of 31.87 ± 0.19% to 34.06 ± 1.45% during pre-monsoon, 44.75 ± 2.25% to 47.94 ± 1.42% during monsoon, and 37.63 ± 0.86% to 42.27 ± 1.50% during post-monsoon. In the current investigation, a significant increase in moisture content levels was seen in all seasons, with pre-monsoon values being lower and monsoon values being greater in all the test sites. During the experimental period, the mean value of moisture content (%) varied between (30.80±0.77 to 50.49±1.45) in pre-monsoon, (66.18±1.24 to 44.75±2.25) in monsoon and (59.01±0.97 to 37.63±0.86) in post monsoon. Looking at the overall pattern, it can be inferred that the soil moisture content rises after the monsoon, progressively falls during the post-monsoon, and displays low values before the monsoon.

In all the sampling areas, the monsoon season exhibited high moisture content, which could be attributed to the high rate of rainfall (615mm). Soil moisture seasonality is consistent with rainfall patterns; thus, soil moisture content is maximum during the rainy season and minimum in summer. Soil physiochemical parameter studies by Singh and Kashyap (2007), and Li et al., (2016) at different ecosystems, also recorded maximum soil moisture content during the rainy season and minimum soil moisture content during the summer season. The moisture content of the S1 soil sample is higher than other soil samples with an average value of 66.8% to 50.89%. The S1 study site which is the forest area (virgin soil) shows higher moisture content because the rate of transpiration and evaporation from open soils exceeds that from forest soils. As a result, during the dry summer and winter seasons, forest soils have more moisture than equivalent open soils (Engler, 1919). According to Osuji and Nwoye, (2007) and Edori and Iyama, (2017) high moisture content is due to de-aeration which displaces air in the soils. Seasons across India play a significant role in controlling the variance in moisture content over various geographical regions. The soil moisture content of the present study is represented in Figure No:4.2.

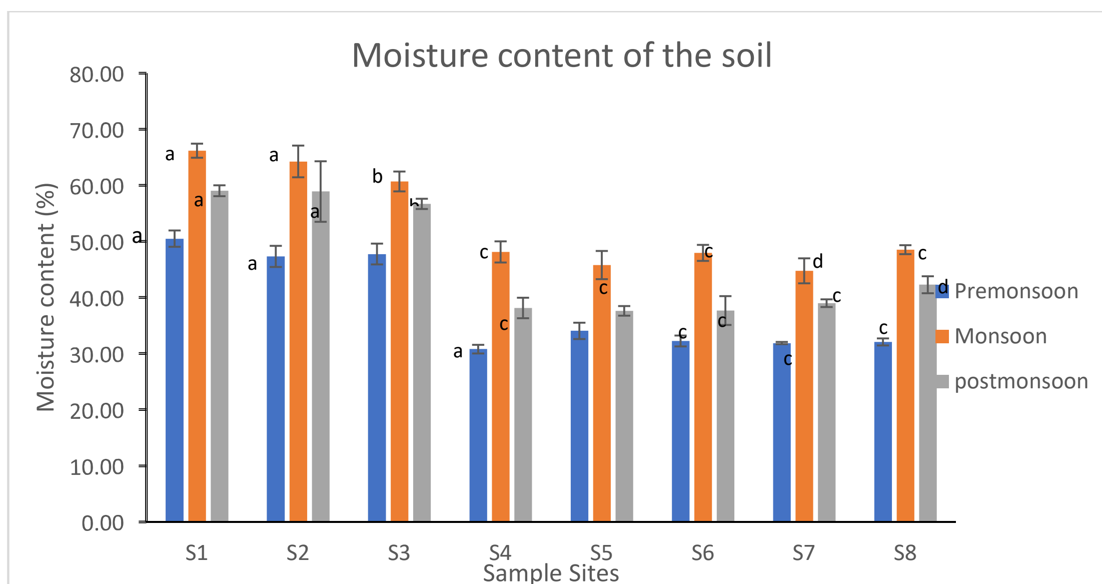


Figure 4.2: Seasonal variation of moisture content of soil in eight study sites. Values are expressed in Mean±SD. P value (≤0.05). Means that do not share a letter are significantly different.

4.1.3 Electrical Conductivity of Soil

The total amount of dissolved salts in a soil sample is expressed in terms of electrical conductivity and measured using a conductivity meter. Soil Electrical Conductivity (EC) is a significant parameter that affects soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity, and subsoil features (Solanki and Chavda, 2012). EC is well-established to be a good indicator of dissolved solids (Singare et al., 2011). The growth and output of crops are influenced by the EC of the soil. Conductivity more than 0.8 ds/m has no negative effect on the crop, 0.8-1.6 ds/m is crucial for salt-sensitive crops, 1.6-2.5 ds/m is critical for salt-tolerant crops, and 2.5 ds/m is most harmful to most crop plants (Gupta, 2000). If the EC is less than 1 (dS/cm), the soil is normal; 1-2 (dS/cm) is important for germination; 2-3 (dS/cm) is critical for the growth of salt-sensitive crops; and EC values of more than 3 (dS/cm) are extremely harmful to crops (Deshmukh, 2012). Boulding, (1994) classified soils based on their EC (µS/cm) as non-saline (<2), moderately saline (2-8), very saline (8-16), and extremely saline (>16).

The EC of the present study varies at the range of 0.44 ± 0.26 $mScm^{-1}$ to 0.75 ± 0.02 $mScm^{-1}$ during pre-monsoon, 0.24 ± 0.01 $mScm^{-1}$ to 0.63 ± 0.01 $mScm^{-1}$ during monsoon, and 0.27 ± 0.01 $mScm^{-1}$ to 0.69± 0.02 $mScm^{-1}$ during post-monsoon. Site S1 shows the EC of the soil at the range of 0.65 ± 0.03 $mScm^{-1}$ during pre-monsoon,

0.50 ± 0.02 $mScm^{-1}$ during monsoon, and 0.58 ± 0.01 $mScm^{-1}$ during post-monsoon. The EC of the site S2 and S3 varies in the range of 0.75 ± 0.01 to 0.80 ± 0.01 during pre-monsoon, 0.63±0.01 during monsoon, and 0.69 ± 0.01 during post-monsoon. Site S4 marked the EC value of the soil as 0.45 ± 0.01 during pre-monsoon, 0.26 ± 0.0 during monsoon, and 0.32 ± 0.01 during post-monsoon. In the sites S5 to S8 the EC value of the soil is in 0 the range of 0.44 ± 0.01 to 0.53 ± 0.02 during pre-monsoon, 0.24 ± 0.01 to 0.35 ± 0.02 during monsoon, and 0.27 ± 0.02 to 0.41 ± 0.01 during post-monsoon.

In the present study, the EC of the soil is higher during pre-monsoon, followed by post-monsoon and monsoon seasons. This could be attributed to the decrease in rainfall during these two seasons. The decrease in rainfall increases the number of electrolytes (salts) on the soil surface resulting in the rise of soil salt content which enhances the soil EC (Paine, 2003). The increase of EC of soil during pre-monsoon could be attributed to the rise in temperature in pre-monsoon. Naeem and Begum (2020) in a similar research on soil at a different ecosystem reported that EC of the soil increases with the temperature rise.

The application of fertilizers at study sites during the soil collection might be one of the reasons for the high EC in soil samples. Trivedy, (1986) observed that the EC of the soil shows a dramatic increase during the monsoon season due to the presence of specific contaminants. The study conducted by Yadav *et al*., (2019) in the soil samples from Pune shows that EC of the soil increases during post-monsoon than in pre-monsoon due to the presence of high water content. The soil EC also increases with an increase in the water content of the soil (Abhilash *et al*., 2014; Dev *et al*., 2017). Therefore, based on the above findings, it can be inferred that the EC of the soil is dependent on the temperature, water content, and application of pesticides. The EC of the soil samples is represented in Figure No: 4.3

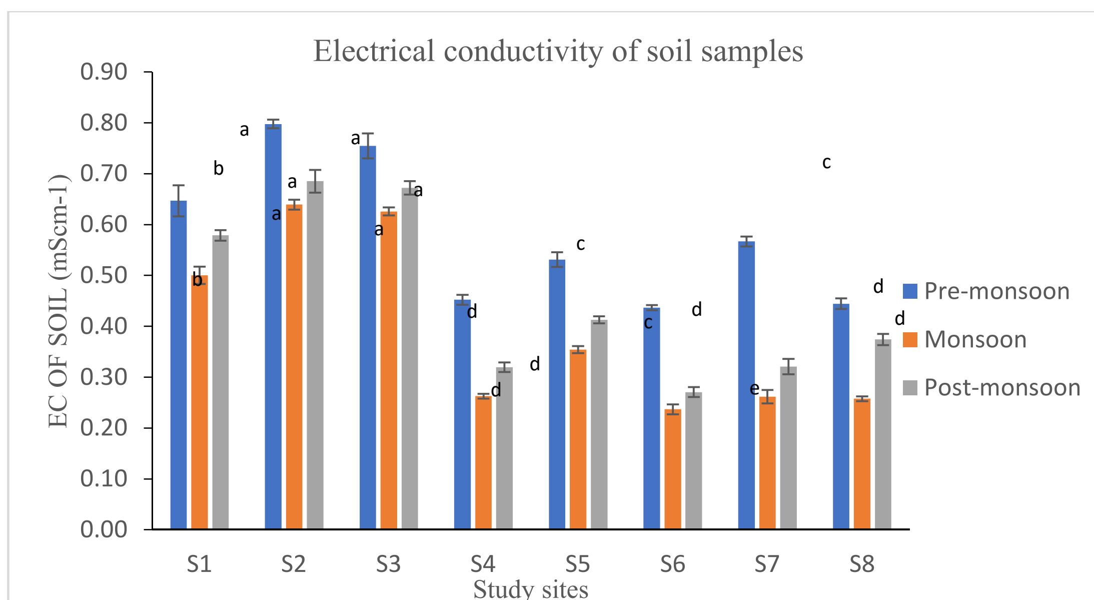


Figure 4.3.: Seasonal variation of Electrical conductivity (EC) in eight study sites. Values are expressed in Mean±SD. P value (≤0.05). Means that do not share a letter are significantly different.

4.1.4 Organic Carbon in Soil

Soil Organic Carbon (SOC) is a vital and measurable component of Soil Organic Matter (SOM). When terrestrial ecosystems change, soil carbon reacts and is sensitive to it. It is a crucial indicator of the health and quality of the soil. Most organic matter is located near the soil surface. Soil includes organic carbon in the form of complex combinations; 90% of soil nutrients exist in the organic matter as compound mixtures (Viscarra et al.,2014).

In this study, the SOC content was higher during the monsoon, followed by post-monsoon. The lowest value of SOC was observed during the pre-monsoon season. Throughout the investigation, soil samples from S1 (forest soil) have a high organic carbon concentration. In site S1, the SOC was 7.56±0.11% during pre-monsoon, 8.7±0.25% during monsoon, and 7.98±0.30% during post-monsoon season. The SOC content of sites S2 and S3 varies in the range of 2.51-3.17% and 2.34-4.54% respectively. In site S4 organic carbon content recorded the maximum mean value of 5.99±0.14% during the monsoon season and a minimum mean value of 3.65±0.12% during the pre-monsoon season. In site S5 the organic carbon of the soil was 3.49±0.09 % during pre-monsoon, 5.99 ± 0.14% during monsoon, and 4.39 ± 0.54% during post-monsoon. The site S6 recorded a maximum SOC value of 6.183 ± 0.16% during monsoon and recorded a minimum SOC content value of 4.428±0.14% during

pre-monsoon. In site S7 the SOC content value was 6.175±0.07% during monsoon, and 4.627 ± 0.14% during pre-monsoon season. Site S8 recorded the maximum SOC content value of 6.195 ± 0.09% during monsoon and recorded the minimum SOC content value of 4.22 ± 0.07% during the pre-monsoon period.

Site S1 shows the highest SOC content throughout the study period in comparison to other study sites because forest soils contain a diversity of soil microorganisms and so can retain more carbon than soils from other land uses (Babur and Dindaroglu, 2020). The main element that influences the soil organic carbon content is seasonal variations. According to Herold et al., (2014), SOC rises with increasing precipitation and temperature fall which is one of the reasons for the high value of SOC during monsoon season. The rise in rainfall enhances plant growth which increases organic matter in the soil. This results in less oxidation of organic materials during monsoon and post-monsoon seasons, which leads to high SOC content during these seasons. According to Allen *et al*., (2005), low rates of decomposition relative to production in cold and wet climates led to an increase in organic carbon content during these seasons relative to pre-monsoon. The decrease in the soil carbon content during the pre-monsoon season could be attributed to moderate temperature and low precipitation during that period.

The investigation reflects that soil organic carbon content is highly influenced by seasonal variations. Boerner *et al*., (2005) analysed the SOC content of three mixed oak woodland systems in Ohio and discovered statistical variations of soil SOC in a range of different seasons and plant species. Stoyan *et al*., (2000) studied the soil organic content of wheat fields under Michigan research and they also reported that SOC content varies significantly with different seasons. These studies suggest that seasonal variations highly influence the SOC as is inferred in this study. Luizao et al., (1992) studied the SOC of the soils in the tropical Amazon jungle and reported that SOC shows no discernible seasonal fluctuations. The SOC content of the present study is presented in Figure 4.4.

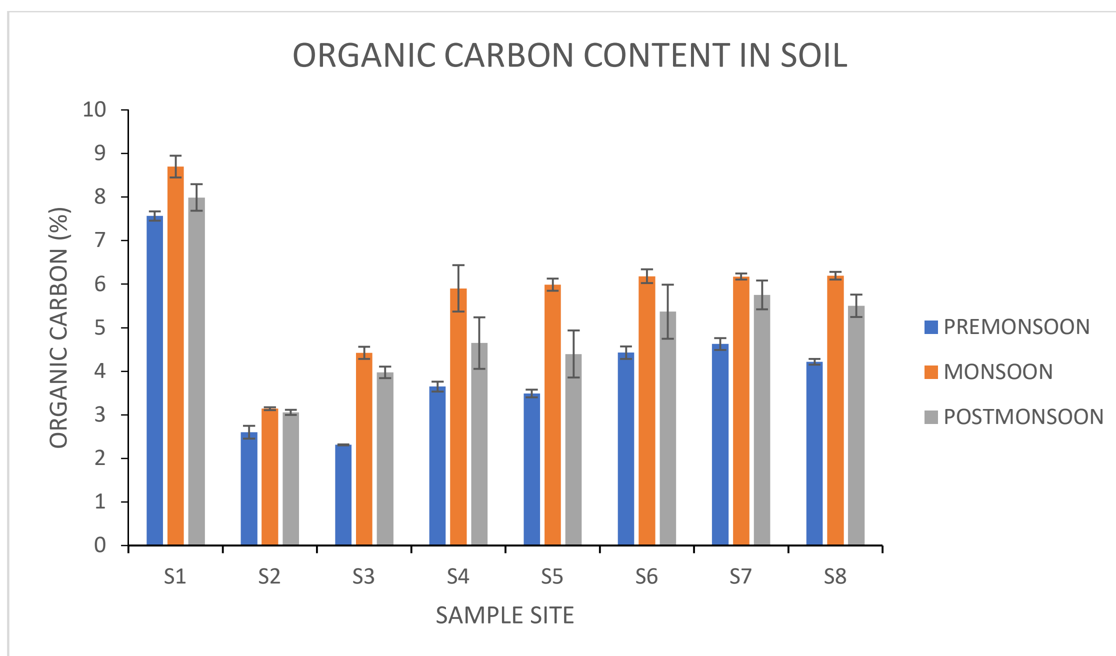


Figure 4.4: seasonal variation of Soil Organic Carbon in eight study sites. Values are expressed in Mean±SD. P value (≤0.05). Mean values that do not share a letter are significantly different.

4.1.5 Soil Nitrogen

Nitrogen promotes above-ground plant growth and gives the leaves a rich green hue. It is absorbed by plant roots in the form of NO_3 and NH_4. Nitrogen supply, both organic and inorganic, is also critical for increased moisture conservation (Agarwal et al., 2005). Most of the nitrogen in the soil is generally found in complicated combinations with organic materials, which plants cannot directly utilize. The available nitrogen values are expressed in the unit kilogram per hectre (kg/ha). For determining the available nitrogen pool in soil, the alkaline permanganate method has been utilized.

This study indicates noticeable fluctuations in the quantity of soil total nitrogen, during different seasons. In site S1, total nitrogen in soil was 658.53±4.81kg/ha during post-monsoon, 614.33±7.53 kg/ha during pre-monsoon, and 481.50±1.83kg/ha during monsoon. The value of available nitrogen in site S2 was 376 ± 2.47kg/ha during post-monsoon, 318.17 ± 6.18 kg/ha during pre-monsoon, and 201.93 ± 2.03kg/ha during monsoon. In site S3 the maximum value of available nitrogen 415.37 ± 4.63 was recorded during the post-monsoon season and the minimum value of available nitrogen 251.85 ± 2.58 was recorded during the monsoon season. In the

transition area (S4), the available nitrogen in the soil was 418.16 ± 6.97 during post-monsoon, 391.91 ± 10.79 during pre-monsoon, and 365.93 ±13.7 during monsoon.

The nitrogen content recorded was low during the monsoon season at all the study sites. This is because it is highly leachable by nature. Soil nitrogen is noted as a limiting nutrient in tropical soils; leaching, surface runoff, and denitrification leads to the increasing loss of nitrogen from soil (Maithani et al., 1998; Cai et al., 2002). However, it needs to be mentioned that there are studies of soil conducted in different ecosystems where the value of total nitrogen is high during monsoon season. For example, Jain and Singh, (2014) examined accessible nitrogen in soils of Madhya Pradesh, and the greatest total nitrogen concentration was recorded in October, while the lowest was in April.

The nitrogen concentration of the soil samples of S5 to S8 is slightly higher than that of the other samples (S2 and S3) throughout the year. The highest nitrogen content in the sites S5 to S8 may be attributable to the use of nitrogen fertilizers at the sampling locations and the delayed breakdown of organic matter from dead plants that have accumulated on the top layer in cold and water-saturated soils.

The available nitrogen in soil samples of S1(forest soil) is higher than that in S5, S6, S7, and S8. This could be attributable to the usage of pesticides in these sites. In sites S5 to S8, synthetic pesticides were applied for the control of pests. Pesticide application hinders the availability of nitrogen content in the soil. Singh and Gulati, (1972) reported that the amount of ammoniacal nitrogen in pesticide-treated soils was lower when compared with control soil samples without pesticides. A study by Seed and Idriss, (1973) inferred that higher concentrations of monocrotophos and methidathion reduced ammonification in soil samples. Murugan *et al*. (1977) noticed a reduction in ammoniacal nitrogen in soil treated with pesticides. Vig *et al*., (2001) found that nitrogen levels are lower in monocrotophos-treated soils when compared with untreated soil samples. Sardar and Kole, (2005) reported that the use of chlorpyrifos lowered nitrogen availability in the soil samples. Fox et al., (2007) found that synthetic pesticides have been shown to severely limit nitrogenase activity, resulting in a reduction in available nitrogen in soil samples. Such research findings arrive at the conclusion that the continuous usage of pesticides reduces the available nitrogen in the soil. It is also significant that there have been studies of soil on

different ecosystems like that of Nair and Taibudeen, (1973), Das and Mukherjee, (2000), and Das *et al*., (2013) that reported that the application of pesticides has no negative consequences on available nitrogen at different concentration. In sites S2 and S3 (where natural plant-based pesticides are used) the value of available nitrogen in the soil was lower when compared to other sites. The decrease in available nitrogen value in organic plots might be due to crop absorption, immobilization by microorganisms, and loss through volatilization (Defoer, 2000).

The available nitrogen in the soil samples of the present study is represented in Figure 4.5.

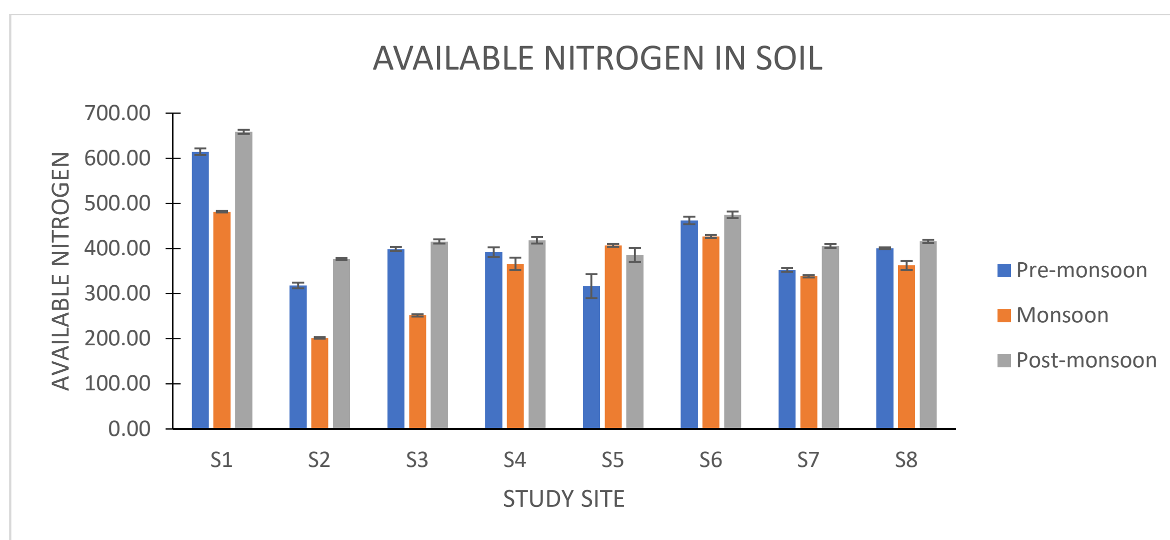


Figure 4.5 : Seasonal variation of Available nitrogen in eight study sites. Values are expressed in Mean±SD. P value (≤0.05).

4.1.6 Soil Phosphorous

Phosphorus is available in the soil in various forms and combinations. Bray's method is used to quantify accessible phosphorus in acidic soil. Olsen's method determines phosphorus using a spectrophotometric approach at a wavelength of 635nM.

. The total content of phosphorus in the soil in all the study sites increased during the monsoon and decreased during the pre-monsoon. In site S1 the phosphorous in soil was 37.83±0.24 during pre-monsoon, 40.89± 0.46 during monsoon, and 39.57 ±1.16 during post-monsoon. Site S2 recorded a maximum soil phosphorous value of 35.13 0.71 during monsoon and a minimum of 27.50± 1.05 during pre-monsoon. In S3, the phosphorous soil was 51.15 ±1.09 during monsoon, 46.66± 2.6 during post-monsoon, and 44.12± 1.04 during pre-monsoon. Site S4 marked the maximum soil phosphorous

value of 41.56 ±0.81 during monsoon and minimum mean phosphorous value of 31.44± 0.74 during pre-monsoon. Phosphorous values of S5 were 36.94± 0.95 during pre-monsoon, 46.50± 1.75 during monsoon, and 39.88 ±2.21 during post-monsoon. In S6, the soil phosphorous value was 32.81 ±4 during pre-monsoon, 45.72 ±0.45 during monsoon, and 42.73± 1.75 during post-monsoon. Phosphorous values of site S7 were 23.76± 1.9 during pre-monsoon, 32.10 ±1.07 during monsoon, and 29.07± 1.77 during post-monsoon. Site S8 marked the maximum phosphorous value of 45.88 ±0.84 during monsoon and the minimum value of 37.24 ±1.62 during pre-monsoon.

In the control sample S1, the phosphorous levels were low in comparison with other study sites; this could be attributed to the continuous absorption of available phosphorous by microorganisms in the forest soil. The phosphorous levels were found to be high during the monsoon and post-monsoon season. Increased phosphorus concentration during and after the monsoon is the result of phosphate ion absorption into soils and the increased rainfall. The phosphorous level of sites S3 and S4 was high when compared to sites S5, S6, S7, and S8. This could be attributed to the accumulation of phosphorous because of the application of chemical fertilizer at these sites. The level of phosphorus was low during pre-monsoon season for the dryness of the soil causes the drop in phosphorous levels. Havlin et al., (2016) reported that dry soils contain less available phosphorus.

Compared to the sites S1 to S4, the soil samples from S5 to S8 have the least phosphorus content. This could be attributed to the application of pesticides here as indicated by previous studies: Sardar and Kole (2005) observed that the usage of pesticides inhibits phosphate solubilizing bacteria which alters the activity of phosphatase enzyme and finally leads to a decrease in the amount of available phosphorous in soil. Hussain et al., (2009) reported that pesticide-contaminated soils showed the inactivation of phosphorus-solubilizing bacteria. However, there are some research reports in different ecosystems that have inferred that pesticides have no negative effect on soil phosphorous status (Tu, 1970; Nair and Taibudeen, (1973).

Microbial activity also enhances phosphorous availability in soil. The increased proliferation and activity of P solubilizing microorganisms, as well as increased acid and alkaline phosphatase activity, resulted in enhanced solubilization of insoluble inorganic phosphate compounds and mineralization of organic P. The moisture

content is high during monsoon, and this increases the microbial population. This, in turn, resulted in an increase in soil P availability during the monsoon season (Das and Debnath, 2006; Das *et al.,* 2012). This study reveals that soil phosphorous level is affected by seasonal fluctuations, use of chemical fertilizers, and pesticides. The available phosphorous content in the soil is represented in Figure 4.6.

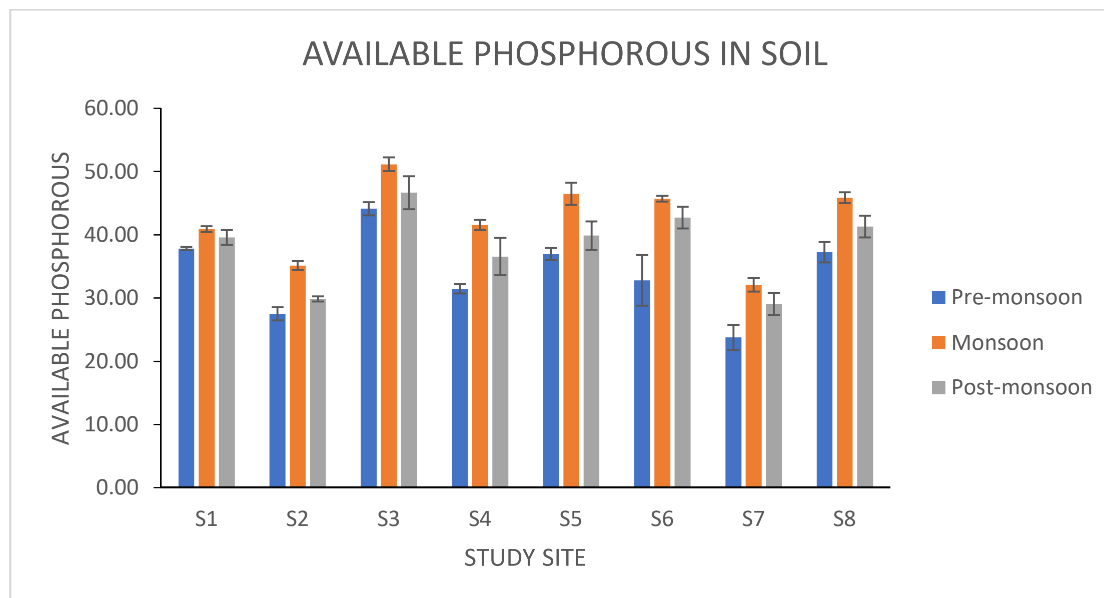


Figure 4.6 : Seasonal variation of Available phosphorous in the soil in eight study sites. Values are expressed in Mean±SD. P value (≤0.05).

4.1.7 Soil Potassium

Potassium is a macro element that is required in substantial amounts by all living species for appropriate plant growth and development. Plants can only absorb potassium from soil solutions, and it is entirely in the form of K^{+} ions. Its availability is determined by both the potassium dynamics and the overall potassium content. In this study, the available potassium in the soil was calculated using a digital flame photometer.

Site S1 recorded the maximum mean soil potassium value of 388.95 ±1.57 during monsoon and minimum mean soil potassium value of 388.62 ±0.02 during pre-monsoon. In S2, the soil potassium recorded the maximum mean value of 307.13± 2.43 during monsoon and minimum mean value of 297.48± 3.76 during pre-monsoon. Soil potassium concentration in S3 was 268.80± 0.96 during pre-monsoon, 277.76 ±0.83 during monsoon, and 273.48 ±2.9 during post-monsoon. In S4 the soil potassium level was 324.79± 0.31 during pre-monsoon, 333.26 ±1.73 during

monsoon, and 328.4 ±2.08 during post-monsoon. S5 recorded the soil potassium maximum mean value of 360.85 ±2.04 during monsoon and a minimum mean value of 353.61 ±2.6 during post-monsoon. The potassium levels of S6 were maximum during monsoon with a mean value of 333.21 ±3.25, and minimum during pre-monsoon with a mean value of 325.54± 0.36 kg/ha. In S7 the soil potassium value was 259.12± 0.69 during pre-monsoon, 270.18 ±1.07 during monsoon, and 264.92± 2.7 during post-monsoon. Site S8 recorded the maximum mean potassium value of 170.72± 1.35 during monsoon and a minimum mean value of 158.76 ±2.15 during pre-monsoon.

The increased concentration of available potassium in the soil during the monsoon was linked to increased soil moisture and the degradation of crop wastes, which releases potassium into the soil. The solubilization of unavailable forms of potassium from potassium-bearing minerals such as micas and illite, as well as by microorganisms excreting organic acids that either directly dissolve rock potassium or chelate silicon ions to bring the potassium into soils (Groudeva and Groudev, 1987). The crop uptake and potential potassium fixation because of the drier soil conditions in the summer were attributed to the decrease in total potassium during the pre-monsoon. Fardous *et al*., (2011), reported that potassium is crucial for plants to survive in stressful situations.

Sardar and Kole, (2005) reported that the treatment of soil with chlorpyrifos reduced the availability of nitrogen, phosphorous, and potassium in soil samples. Aikpokpodion *et al.*, (2010) noticed an uneven pattern of potassium distribution in soil when treated with the pesticide endosulfan in their research sites. Sebiomo *et al*.,(2012) studied the effects of atrazin, glyphosate, and primeextra on soil samples. They reported that the application of these pesticides decreased the potassium content in soil samples. Sauwa and Yakubu, (2013) found that soil supplemented with pesticides dichlorvos, karate, and phoskill had lower levels of nutritional components than untreated soils. It can be inferred from such studies that the amount of potassium that is readily available in soils is impacted by pesticide application. When compared to the other sample sites, the soil samples of S7 and S8 had the lowest potassium level, which can be empirically attributed to the continuous application of synthetic pesticides in the study sites. The soil potassium values during different seasons are represented in Figure 4.7.

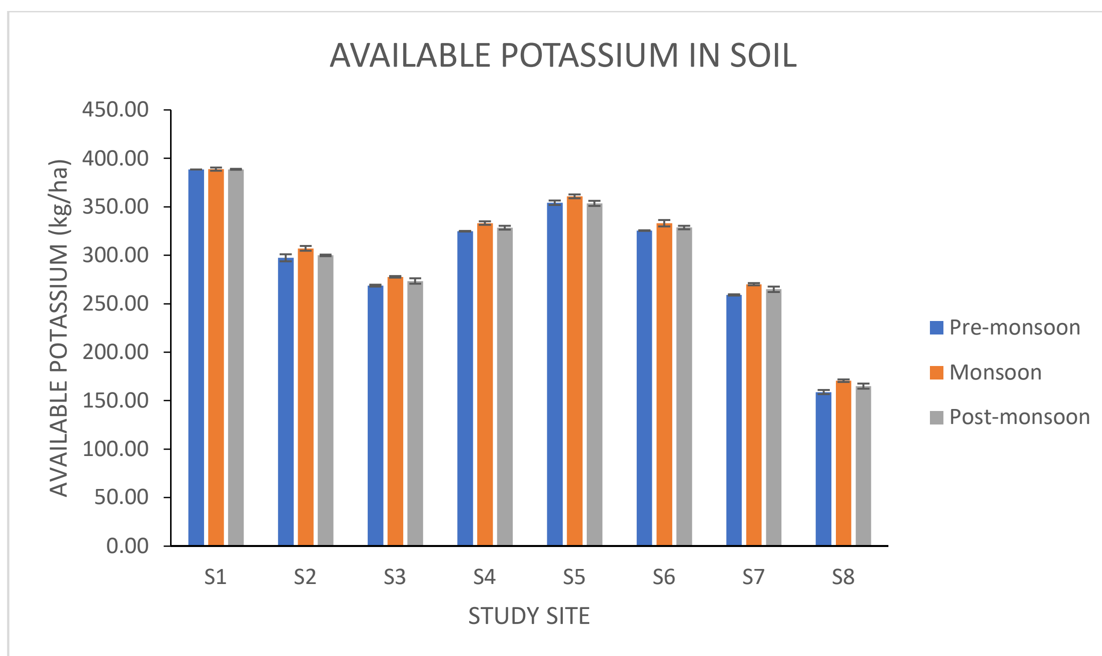


Figure 4.7 : Seasonal variation of Available potassium in eight study sites. Values are expressed in Mean±SD. P value (≤0.05).

4.1.8 Soil Texture

Soil texture is one of the most permanent characteristics of soil, and it is only slightly affected by different farming approaches. Soil texture has a direct impact on soil-water relations, aeration, and root penetration. It also has an impact on the nutritional condition of the soil. Soil is classified into textural groups based on the proportion of distinct particle sizes of sand, slit, and clay. In this study, soil texture was analysed using the hydrometer method.

The soil texture of the study sites S1, S2, S3, S4, S5, S6, and S7 were sandy clay loam in nature with the highest sand percentage (sand>clay>slit). The soil texture of S8 was sandy loam with zero percent of slit. The presence of all three types of soil materials, as well as the presence of humus, enhances the quality of the soil and makes it ideal for the cultivation of crops. According to Molepo *et al.,* (2017), in comparison to sandy soil, clay-textured and wet soil is highly conductive.. Soil texture had a significant impact on the microbial population in all samples, and it is one of the most critical variables influencing the soil microbial community. The pH and calcium levels are also higher in sandy clay loam soils in comparison to other soil types.

Soil texture influences the retention rate of pesticides in soils. Menon *et al.,* (2004) reported that pesticides (chlorpyrifos and quinalphos) had a bigger inhibitory impact

in loamy sand than in sandy loam soil. In loamy sand soils, the bioavailability of pesticides is higher due to the low clay content in them. The rate of pesticide degradation is also affected by the texture of the soil. Researchers (Kumar *et al.*, 2012) also identified a link between pesticide adsorption, degradation, and soil texture. They investigated the adsorption of endosulfan isomers on various soils and discovered that pesticide adsorption was highest in clay soil, followed by composted soil and red soil, while endosulfan degradation was highest in sandy soil, followed by red soil, composted soil, and clay soil, implying that pesticide degradation is inversely proportional to pesticide adsorption, which is closely related to soil texture. Cycon *et al.*, (2013) analysed the degradation rates of different pesticides (chlorpyrifos, fenitrothion, parathion) and found that the rate of degradation is higher in sandy loam soil when compared to other soil types (sandy soil and silty soil). It can thus be deduced that the rate of pesticide degradation is higher in sandy loam soil. The soil texture of the study samples is represented in Table 4.2.

Table 4.2 :- Soil texture (%) of eight different study sites

Sample Code	**Clay (%)**	**Slit (%)**	**Sand (%)**	**Texture Class**
S1	30.21	8	61.79	Sandy clay loam
S2	26.16	2	71.84	Sandy clay loam
S3	26.16	2	71.84	Sandy clay loam
S4	28.16	3	67.84	Sandy clay loam
S5	26.16	6	67.84	Sandy clay loam
S6	26.16	2	71.84	Sandy clay loam
S7	20.16	2	77.84	Sandy clay loam
S8	16.16	0	83.84	Sandy loam

4.1.9 Soil Micronutrients

The micronutrients in the soil analysed for this study were copper, zinc, manganese, and iron. In S1 the value of copper varies at the range of 5.61 to 7.97 ppm, and the value of zinc varies at the range of 5.2 to 7.5 ppm. In this site, the value of manganese varied in the range of 2.1 to 0.8 ppm, and the value of iron was 35.6 to 41.1 ppm. Site S2 recorded the value of copper in the range of 4.85 to 5.12, and the value of zinc in the range of 1.15 to 1.28. The value of manganese in this site varies at the range of 21.02 to 26.01, and the value of iron at the range of 25.61 to 35.9 ppm. The values of copper in S3 were in the range of 3.87 to 6.9 ppm, and the values of zinc were in the range of 0.21 to 0.51 ppm. The values of manganese in S3 were 1.8 to 4.7 ppm, and the values of iron were in the range of 25.8 to 30.21 ppm. In S4 the value of copper was at the range of 3.8 to 8.74 ppm, and the value of zinc was in the range of 0.86 to 1.2 ppm. The values of manganese were in the range of 21.81 to 26.9 ppm, and the values of iron were in the range of 41.25 to 47.11 ppm in S4. In site S5 the value of copper was in the range of 3.2 to 8.7 ppm, and the value of zinc was in the range of 0.21 to 1.7 ppm. The manganese in soil in the site S5 was in the range of 24.2 to 37.8 ppm, and the iron in the soil was in the range of 40.25 to 47.11 ppm. In S6 the value of copper was in the range of 4.12 to 7 ppm, and the value of zinc was in the range of 0.58 to 2.4 ppm. The value of manganese was in the range of 3.95 to 0.43 ppm, and the value of 40.12 to 41.25 ppm in the same site. Site S7 recorded the maximum value of copper in the range of 3.45 to 7.15 ppm, and the value of zinc was in the range of 0.32 to 2.65 ppm. The value of manganese was in the range of 22.5 to 26.7 ppm, and the value of iron was in the range of 22.15 to 25.31 ppm at the same site. In site S8 the value of copper was in the range of 3.89 to 8.21 ppm, and the value of zinc was in the range of 0.21 to 2.21 ppm. In the same site, the value of manganese was in the range of 45 to 32.56 ppm, and the value of iron was in the range of 38.75 to 40.65 ppm. The values are depicted in the Table 4.3.

S5, S6, S7, and S8 had higher concentrations of copper in soil than other sites. This could be attributed to the usage of excessive quantities of pesticides, fungicides, and manure in these sites. The variation of concentration of copper in the soil harms agricultural system by altering the soil fertility levels. The excess concentrations of copper in soil are extremely hazardous to microorganisms. Paul *et al*., (2013) reported that the usage of herbicide 2-4D alters the copper levels in soil and it results in the cellular immobilization of the microorganisms.

The level of zinc in the sites S5, S6, S7, and S8 were lower in comparison with other four study sites. This could be attributed to the application of pesticides altering the level of zinc in soil. A study by Sebiomo *et al*., (2012) found that the application of atrazine, glyphosate, and prime extra increased the level of zinc in soil. Paul *et al*., (2013) reported that the use of herbicide 2-D increased the amount of accessible zinc in soil samples. Such studies affirm that the usage of pesticides will alter the levels of zinc in soil.

The level of manganese was found to be higher in sites S2, S3, S7, and S8 in comparison with the other four study sites. This could be attributed to the usage of fertilizers and pesticides in these sites. Qian *et al*., (2019) reported that the application of fertilizers and pesticides will raise the concentration of elements like lead, copper, and manganese in groundwater and soils. The levels of iron in sites S4, S5, and S6 were relatively higher than the other five study sites. This may be due to the application of pesticides on these sites at the time of soil sample collection. Previous studies have emphasised that the usage of pesticides will alter the level of micronutrients in the soil: Schreinemachers and Ghio (2016) found that glyphosate forms complexes with iron and alters its level in the soil..

Table 4.3 The micronutrient (Range) ppm of soils of the period 2018-2019.

Sample Sites	Micronutrients (ppm)			
	Copper	Zinc	Manganese	Iron
S1	5.61 – 7.97	5.23 – 7.51	2.16 – 0.86	35.6 – 41.17
S2	4.85 – 8.12	1.15 – 1.28	21.02 – 26.01	26.61 – 35.9
S3	3.87 – 6.98	0.21 – 0.57	1.89 – 4.75	25.8 – 30.21
S4	3.87 – 8.74	0.86- 1.27	21.81 – 26.98	41.25 – 47.11
S5	3.21 – 8.74	0.21 – 1.70	24.28 – 37.87	40.25- 47.11
S6	4.12 – 7.07	0.58 – 2.49	3.95 – 0.43	40.12 – 41.25
S7	3.45 – 7.15	0.32 – 2.65	22.56 – 26.78	22.15 – 25.31
S8	3.89- 8.21	0.21- 2.21	23.45 – 32.56	38.75 – 40.65

4.1.10 Bacterial Population in Soil

Bacterial population in soil exhibited the highest growth rate during the monsoon season and the lowest during the pre-monsoon season. The mean bacterial population was estimated in two different dilutions (10^5 and 10^6). The annual mean population of heterotrophic bacteria in this study varied from 36.5 ± 4.7 to 135.6 ± 5.8 in the pre-monsoon, 68.33 ± 3.05 to 246.6 ± 5.29 in the monsoon, and 84.66 ± 3.05 to 260.6 ± 6.02 in the post-monsoon in the dilution factor 10^5 (Table 4.4 and 4.5). The bacterial counts in ranged from 19 ± 3 to 130 ± 5 in the pre-monsoon, 54 ± 5.29 to 177.6 ± 2.08 in the monsoon, and 66.33 ± 4.7 to 215 ± 5 in the post monsoon at the dilution factor 10^6.

Site S1 exhibited the maximum bacterial population during all the seasons, with the maximum bacterial population being 260.6±6.02 at dilution 10^5 and 215±5 at dilution 10^6 during post-monsoon. This could be attributed to the high moisture content and SOC of forest soils that enhance the growth of microorganisms. Forest soils have a stable and thick cover of plant matter making them rich in organic carbon compounds and microbial biomass (Bacmaga *et al.,* 2022). In all eight study sites, the bacterial mean population was highest during the monsoon season. This may be due to the high moisture content and nutrient availability during the monsoon season as indicated in recent studies. Jamieson *et al*., (2002) in their study of different ecosystems stated that bacterial growth is higher in cool and moist conditions. Vegetation type, weather conditions, and soil characteristics are the factors that affect bacterial survival (Lipson and Schmidt, 2004; Brockett *et al.,* 2012).

The coliform count was compared between seasons and between dilutions. The interaction between season and dilution was also tested. For this, a two-way ANOVA was carried out and the pairwise comparison was done using the Least Significant Difference (LSD) test. Table 4.6 shows that all the F-values were found to be significant at 0.01 level as the P-values are less than 0.01. A significant F-value (5.203) for season indicates that there exists a significant difference in coliform count between seasons. Pair-wise comparison using the LSD test in the case of between overall seasons averaged over two dilutions shows that coliform count is significantly higher in the post-monsoon season compared to the other two seasons and no significant difference was noted in coliform count in the pre-monsoon and monsoon seasons.

Table 4.4:- Bacterial population of eight different sites during three seasons at two different dilutions (2018-2019). Values are expressed as Mean ± SD.

Sample no	Season	Bacterial population (CFU/gm)	
		Dilution 10^{-5}	Dilution 10^{-6}
S1	Pre-monsoon	135.6±5.8	130±5
	Monsoon	246.66±5.29	177.6±2.08
	Post-monsoon	260.6±6.02	215±5
S2	Pre-monsoon	88±2.64	76.6 ±2.08
	Monsoon	135.6 ±5.85	107 ±4.35
	Post-monsoon	136.66 ± 7.63	118.33 ± 3.5
S3	Pre-monsoon	69 ±3.6	58 ± 3.5
	Monsoon	132± 8.8	121.3 ±5.68
	Post-monsoon	133.66 ± 7.23	115 ± 5.29
S4	Pre-monsoon	68.3 ± 6.5	52.3 ±5.13
	Monsoon	111.3 ± 9.07	94 ± 7.8
	Post-monsoon	121.33 ± 4.16	96.6 ±7.57
S5	Pre-monsoon	36.5± 4.7	26.33 ± 1.5
	Monsoon	81 ± 3.6	66 ± 2
	Post-monsoon	84.66 ± 3.05	67.33 ±5.03
S6	Pre-monsoon	56 ± 4	45± 4
	Monsoon	68.33± 3.05	54 ±5.29
	Post-monsoon	86.66 ± 7.3	66.33 ± 4.7
S7	Pre-monsoon	27.66 ± 2.51	19 ± 3
	Monsoon	77.66 ±2.5	65.33 ± 3.51
	Post-monsoon	89.66 ± 2.5	80 ± 1
S8	Pre-monsoon	60 ±4.35	45.33± 3.51
	Monsoon	78.66 ± 3.5	66 ±4.35
	Post-monsoon	88.66± 3.51	72.33 ±2.51

Table 4.5 the statistical analysis of bacterial population at different seasons

	Mean ± SD			
Dilution	Pre-monsoon	Monsoon	Post Monsoon	Overall dilution
10^5	67.63 ± 6.56^{bB}	56.63 ± 6.79^{bB}	116.33 ± 11.45^{a}	80.19 ± 5.77^{B}
10^6	93.92 ± 8.08^{bA}	125.25 ± 11.53^{aA}	103.88 ± 9.66^{ab}	107.68 ± 5.82^{A}
Overall season	80.77 ± 5.50^{b}	90.94 ± 8.30^{b}	110.1 ± 7.46^{a}	93.94 ± 4.24

F-value between season = 5.203**; P-value = 0.007
F-value between dilution = 13.289**; P-value < 0.001
F-value for interaction between dilution and season = 9.643**; P-value < 0.001

** Significant at 0.01 level
Means having different small letter as superscript differ significantly within a raw (between season)
Means having different capital letter as superscript differ significantly within a column (between dilution)

A significant F-value (13.289) for dilution indicates that there exists a significant difference in coliform count between dilutions. Pair-wise comparison using the LSD test in the case of overall dilution averaged over all seasons shows that coliform count is significantly higher in 10^6 dilutions compared to 10^5 dilutions.

4.1.11 Pesticide Residue in soil samples

Soil samples were examined to determine the pesticide residues in tea gardens at different seasons. Due to the regular application of pesticides for plant crop protection, the soils have been subjected to environmental pollution and these contaminants end up in tea garden soils.

Table 4.6 :- Concentration of ethion, glyphosate, quinalphos and deltamethrin (mg/kg) in soil from eight different sites of Anaimalai hills during pre-monsoon. Values are expressed Mean ± SD.

SAMPLE CODE	ETHION	GLYPHOSATE	QUINALPHOS	DELTAMETHRIN
S1	ND	ND	ND	ND
S2	ND	ND	ND	ND
S3	ND	ND	ND	ND
S4	ND	ND	0.08 ± 0.02	ND
S5	ND	0.16 ± 0.005	ND	ND
S6	0.17 ± 0.005	ND	ND	0.13 ± 0
S7	ND	ND	ND	ND
S8	ND	ND	0.06 ± 0.015	ND

Each of the eight pesticides examined were below the Maximum Residue Level (MRL) throughout the study period. Due to the absence of pesticide application at sites S1 (forest soil), S2, and S3, no pesticide residues were found in any of these locations over the year. In S4 and S8, quinalphos is found in concentrations between 0.08 ± 0.02 and 0.06± 0.01 during pre-monsoon, 0.16 ±0.02 and 0.11 ±0.01 during monsoon, and 0.11± 0.01 and 0.11± 0.02 during post-monsoon. The pesticide residue values were low during pre-monsoon and the highest value was observed during the monsoon.(Table 4.6).

Ethion was discovered in soil samples from site S6 throughout the year in all seasons. This site recorded the maximum concentration of ethion (0.38 ± 0.08) during the monsoon season, 0.17 ± 0.005 during pre-monsoon, and the lowest values (0.15 ± 0.03) during post-monsoon. The low value of ethion during post-monsoon may be due to the increase in bacterial population (86.66 ±7.3 and 66.33± 4.7). The

microorganisms metabolise the pesticides and degrade them, which results in a low rate of ethion during post-monsoon.

Table 4.7 :- Concentration of thiamethoxam, propargite, fenpyroximate, and spiromesifen (mg/kg) in soil from eight different sites during pre-monsoon. Values are expressed as Mean ± SD.

SAMPLE CODE	THIAMETHOXAM	PROPARGITE	FENPYROXIMATE	SPIROMESIFEN
S1	ND	ND	ND	ND
S2	ND	ND	ND	ND
S3	ND	ND	ND	ND
S4	ND	ND	0.053 ± 0.4	ND
S5	ND	0.253 ± 0.02	ND	ND
S6	ND	ND	ND	0.173 ± 0.001
S7	0.026 ± 0.01	0.183 ± 0.01	ND	ND
S8	ND	ND	0.11 ± 0.01	ND

S5 site had the highest concentration of glyphosate (0.26± 0.02) during the monsoon, the lowest concentration (0.16 ± 0.005) during the pre-monsoon, and roughly the same concentration (0.2 ± 0.005) during the post-monsoon. In comparison to the dry season, Organophosphate Pesticides (OPP) levels were higher during the rainy season. According to Daam et al., (2019), the breakdown of pesticides in tropical soils may accelerate during the dry seasons because of increased temperatures. The values are depicted in Table 4.7.

Table 4.8 :- Concentration of ethion, glyphosate, quinalphos and deltamethrin (mg/kg) in soil from eight different sites during monsoon. Values are expressed Mean ± SD

SAMPLE CODE	ETHION	GLYPHOSATE	QUINALPHOS	DELTAMETHRIN
S1	ND	ND	ND	ND
S2	ND	ND	ND	ND
S3	ND	ND	ND	ND
S4	ND	ND	0.16 ± 0.02	ND
S5	ND	0.26 ± 0.02	ND	ND
S6	0.38 ± 0.08	ND	ND	0.71 ± 0.5
S7	ND	ND	ND	ND
S8	ND	ND	0.21 ± 0.01	ND

Low concentrations of pesticides during the dry season may be due to the increased rates of microbial degradation and photodegradation of pesticides in sediments with water content. Stamatis *et al*., [2013] observed the presence of considerably greater organophosphate residue levels in summer (dry season) than in winter (rainy season) in the Achelous River in Greece. The rate of photodegradation is higher during pre-

monsoon which results in the breakdown of pesticides in the dry season (Muskus *et al.*, 2020). The leaching rate of pesticides is higher in sandy loam soils than in other types of soils and results in the low persistence of pesticide residues.. This may be the reason for the low level of pesticide residues in the sites of this study because the texture of the soil samples is sandy loam in nature.

Deltamethrin was found in all the seasons in soil samples collected throughout the year from site S6. Site S6 recorded the greatest levels of deltamethrin (0.71 ± 0.5) during monsoon, followed by 0.526 ± 0.011 during post-monsoon, and 0.13 during pre-monsoon. The low rate of deltamethrin during pre-monsoon may be due to photodegradation. Pyrethroid pesticides will degrade when they are exposed to sunlight (Akoto *et al*., 2013). The high level of deltamethrin in the monsoon season could be attributed to the application of pesticides during that season. This is in consistence with the observation of Saillenfait *et al.,* (2015) who reported that during the rainy season, the farming activities are high, and this increases the level of pesticides in the soil. (Table 4.9).

Table 4.9 :- Concentration of thiamethoxam, propargite, fenpyroximate, and spiromesifen (mg/kg) in soil from eight different sites during monsoon. Values are expressed as Mean ± SD.

SAMPLE CODE	THIAMETHOXAM	PROPARGITE	FENPYROXIMATE	SPIROMESIFEN
S1	ND	ND	ND	ND
S2	ND	ND	ND	ND
S3	ND	ND	ND	ND
S4	ND	ND	0.18 ± 0.03	ND
S5	ND	0.36 ± 0.017	ND	ND
S6	ND	ND	ND	0.2 ± 0.01
S7	0.23 ± 0.051	0.246 ± 0.04	ND	ND
S8	ND	ND	0.263 ± 0.02	ND

Pesticide residues in the sediment samples were substantially greater during the rainy season than during the dry season. Recent studies have indicated that this might be due to the increased runoffs and precipitation of suspended particles that contain pesticide residues: Senyo *et al*. [2016] and Zhang *et al*. [2018] detected increased parathion residues in the rainy season in Ghana's Songhua River and Afram River. Vryzas, (2018) found that the high precipitation rate and surface runoff increase the breakdown of pesticides in the sediments of floodplain. (Table 4.10 and 4.11).

Table 4.10 :- concentration of ethion, glyphosate, quinalphos and deltamethrin (mg/kg) in soil from eight different sites during post-monsoon. Values are expressed Mean ± SD

SAMPLE CODE	ETHION	GLYPHOSATE	QUINALPHOS	DELTAMETHRIN
S1	ND	ND	ND	ND
S2	ND	ND	ND	ND
S3	ND	ND	ND	ND
S4	ND	ND	0.11 ± 0.01	ND
S5	ND	0.2 ± 0.005	ND	ND
S6	0.15 ± 0.03	ND	ND	0.506 ± 0.011
S7	ND	ND	ND	ND
S8	ND	ND	0.11 ± 0.02	ND

Table 4.11:- concentration of thiamethoxam, propargite, fenpyroximate, and spiromesifen (mg/kg) in soil from eight different sites during post-monsoon. Values are expressed as Mean ± SD

SAMPLE CODE	THIAMETHOXAM	PROPARGITE	FENPYROXIMATE	SPIROMESIFEN
S1	ND	ND	ND	ND
S2	ND	ND	ND	ND
S3	ND	ND	ND	ND
S4	ND	ND	0.11 ± 0.01	ND
S5	ND	0.3 ± 0.0	ND	ND
S6	ND	ND	ND	0.14 ± 0.017
S7	0.11 ± 0.02	0.20 ± 0.03	ND	ND
S8	ND	ND	0.21 ± 0.02	ND

Studies by *Arjmandi et al*., [2010] indicate that the insecticide traces were detected in rice fields after three months of pesticide spraying. The soil parameters, time of consumption or application, ambient temperature, all play a significant role in the persistence of pesticide residues. Lahr *et al*., (2016) found that the environmental concentration of the pesticides is dependent upon the type and quantity of pesticides. Seasonal variations will affect the sorts of pesticide residues that are found in the soil samples (Daam *et al.,* 2019). According to Priya *et al*., [2006], the persistence of pesticides was longer in soils with higher clay content, and the breakdown of pesticides was affected by the concentration of clay.

4.1.12 Impact of Soil Parameters on Microbial Population and Fate of Pesticide in Soil

The variation between season and nutrient parameters in the soils was done using one-way ANOVA followed by Duncan Multiple Range Test (DMRT). Results of DMRT shows that these parameters are significantly different between all season.

As the p-value in the case of Potassium is greater than 0.05, it can be inferred that there exists no significant difference in soil potassium between seasons. For all other parameters, F-value is less than 0.01 which shows that there exists a significant difference in the parameters organic carbon, nitrogen, and phosphorous between seasons. In the case of organic carbon, a significantly higher value was observed in the Monsoon season and a significantly lower value was observed in the pre-monsoon season. Phosphorous is also significantly higher in the monsoon season and significantly lower in the pre-monsoon season. However, Nitrogen content is significantly higher in post-monsoon season and significantly lower in monsoon season. The results of the same is given in Table 4.12. In the case of all physiochemical parameters, the p-value was found to be less than 0.01 indicating that there exists a significant difference between seasons in these parameters. Results of DMRT shows that pH, Moisture content, and Electrical conductivity vary significantly between all seasons.

Table4.12. Results of comparison of physiochemical parameters between seasons in the soil of the Valparai region

Variable	Pre-monsoon	Monsoon	Post Monsoon	F-value (P-value)
pH	5.01 ± 0.07^{c}	5.55 ± 0.06^{b}	6.00 ± 0.05^{a}	69.709** (<0.01)
Moisture content	38.32 ± 0.96^{c}	53.28 ± 0.87^{a}	46.16 ± 0.89^{b}	57.376** (<0.01)
Electrical conductivity	0.58 ± 0.02^{a}	0.39 ± 0.02^{c}	0.45 ± 0.01^{b}	31.041** (<0.01)

** Significant at 0.01 level
Means having different small letter as superscript differ significantly within a raw (between seasons)

Table 4.13: Pearson's correlation coefficient for soil physiochemical parameters and bacterial population during pre-monsoon of the year 2018-2019. MC – Moisture content, EC- electrical conductivity, N- Nitrogen, P- Phosphorous, K – Potassium, OC- organic carbon, BC- bacterial count.

	pH	*MC*	*EC*	*N*	*P*	*K*	*OC*	*BC*
pH	1							
MC	0.326653	1						
EC	0.70502	0.86839	1					
N	-0.58466	0.379999	-0.08265	1				
P	-0.39561	0.372348	0.101532	0.349026	1			
K	-0.22775	0.318942	0.143856	0.381095	0.020237	1		
OC	-0.55513	0.318754	-0.06917	0.907538	0.166583	0.373521	1	
BC	-0.16066	0.766507	0.410186	0.735489	0.298062	0.405242	0.615774	1

The bacterial population considerably increased during the monsoon and post-monsoon and significantly decreased during the pre-monsoon. More precisely, during the monsoon and post-monsoon, larger values of the bacterial population were seen in the soils of S1 (forest or virgin soil) on both the dilution factors (246.66 ± 5.29 and 260.6 ±6.02) at 10^5 dilutions and (215± 5 and 177.6± 2.08) at 10^6 dilutions. The persistent and substantial layer of plant material that covers forest soils, makes them rich in microbial biomass and organic carbon molecules. Forest soils are stable, have a substantial layer of plant material on top of them, and are therefore rich in microbial biomass and organic carbon molecules.

The soil pH, moisture content, OC, N, P, and K was high during monsoon and post-monsoon and low during pre-monsoon season. The bacterial population was also observed low during pre-monsoon season in comparison with other two seasons. The bacterial population is lower in the sites S6,S7, and S8 when compared to other sites. These are the sites where synthetic pesticides are applied. The continues application of pesticides decrease the bacterial population in soil samples.

Jing *et al.*, (2021) demonstrated that total N and organic C levels are important indicators of soil quality in forest ecosystems. One of the key factors affecting the diversity of bacteria in this study is pH. Strong correlations were found between bacterial diversity and soil pH, with bacterial diversity being higher moderately acidic to slightly neutral samples and lower in highly acidic samples in accordance with different seasons. In this study, the pH of tea plantation soils falls from acidic to slightly neutral during monsoon is some soil samples and the microbial content is also seen high in this pH level during monsoon (r=-0.16066), and low in pre-monsoon in acidic pH (Table 4.13). According to Jones *et al*., (2009), and Shen *et al.,* (2013), the enrichment of acidophilic bacteria in low-pH soils was typically associated with significant changes in the soil microbial community composition over pH gradients. Similar patterns in bacterial diversity and community composition were also seen in arable soils with an artificial pH gradient. The heavy use of nitrogen fertilizers in agricultural areas may be the main factor causing the soil pH to fall (Guo *et al*., 2010). pH is one of the most important parameters of soil and it has a significant impact on all the physical, chemical, and biological characteristics of soil. The mean bacterial population is weakly correlated (r=0.405) with total potassium. In the present study, pH shows a negative correlation with total nitrogen in the soil (r= -0.58).

According to Aislabie *et al*.,(1995), and Arjmandi *et al.* (2010) soil clay content, pH, cation exchange capacity (CEC), exchangeable cations, moisture, and other factors influence pesticide adsorption in soil. Among the above said factors organic carbon and clay content of the soil have a strong influence on pesticide adsorption. Organic materials in the soil can either reduce microbially mediated pesticide breakdown by promoting pesticide adsorption or increase microbial activity through co-metabolism. Organic matter is the primary sorbent of pesticides in soil due to its high chemical reactivity towards minerals and other organic molecules (Calvet, 1989), and this tendency facilitates pesticide interactions with organic matter, which affects pesticide bioavailability in soil.

Soil moisture is also vital in pesticide degradation because it acts as a solvent for pesticide transport and diffusion and is required for microbial activity. The availability of nutrients and pollutants decreases in dry soil, affecting deterioration, but the diffusion of oxygen decreases in soil with increased water content, affecting microbial activities (Skopp *et al*., 1990). Dureja, (1989) found that monocrotophos degraded faster in moist and flooded soils than in dry soils, and he also reported that the microbial activity enhanced the degradation process of pesticides. The environmental fate of organic contaminants in soils is substantially impacted by soil pH and texture, as well as the presence of organic matter (Awasthi et al., 2000). Degradation occurred more quickly in non-flooded soils. Ultisol (humult) soil has strong alkaline phosphatase activity, which promotes the breakdown of organophosphorus pesticides in soil. It has previously been reported that soil microflora degrades chlorpyrifos more efficiently in neutral pH soil than in acidic soil (Singh *et al*., 2003). Sumit *et al*., (2011) demonstrated that the presence of moisture improves the bioremediation of pesticides by bacterial monocultures. The rate of degradation of pesticides was substantially impacted by changes in soil water content as well as temperature (Ghadiri *et al.,* 2001). An optimum level of organic matter and moisture content is required to maintain an active microbial community in the soil (Rohilla, 2012). High moisture content promotes soil fertility and the proliferation of microorganisms. Organic carbon, and organic matter has a significant impact on the process of pesticide adsorption in the soil [ElShafei *et al.,* 2009]. This is primarily because organic matter and clay particles give the soil more adsorptive sites for pesticide molecules to bind to. Despite the complexity of soil organic matter compositions, it

has been reported that when OM is low (5%), other factors, such as the type and nature of the pesticide, accessibility of its functional groups, inorganic constituents, and soil characteristics may also play a role in the ability of the pesticide to bind to organic matter. The present research findings emphasize on the idea that all the physiochemical parameters of soil have an impact on bacterial population, soil fertility, and pesticide fate in the environment.

4.2 Isolation, Diversity, and Characterisation of Pesticide-Degrading Bacteria from Tea plantation Soils

4.2.1 Isolation and Diversity of bacterial isolates from tea garden soils

The main objective of this study is to isolate indigenous bacteria from the soils of tea gardens that have been sprayed with various pesticides. As a result, it is anticipated that the isolated microorganisms will be more tolerant to pesticides and contribute to their degradation. Bacteria were isolated from tea garden soils where pesticides had been administered for years. As pesticides are regularly sprayed on the soil, soil biota can quickly develop the ability to break down them, providing a carbon source and enough electron donors to soil bacteria (Torres *et al*., 2003).

A total of ten native bacterial isolates were obtained through the enrichment culture technique. The isolated strains from their respective pesticide applied MSM were denoted with code names. The code names and the pesticides they degraded are shown below:

Table 4.14: Name of the pesticides and bacterial isolates obtained, and the code names given for the isolates.

Sl.No	Name of the Pesticides degraded	Code names of bacterial isolates obtained
1.	Deltamethrin	DRNB1
2.	Ethion	EON2
3.	Spiromesifen	SFN1 & SFT1
4.	Thiamethoxam	TXM2
5.	Fenpyroximate	FXE1 & F1T
6.	Quinalphos	Q1T
7.	Glyphosate	GLYB2
8.	Propargite	PTEB2

Three (Q1T, EON2, and GLYB2) of the 10 isolates were found to breakdown organophosphate (Quinalphos , Ethion, and Glyphosate) pesticides, four (FXE1, F1T, SFN1, and SFT1) were found to degrade Fenpyroximate and Spiromesifen, and the remaining three isolates (DRNB1, TXM1, and PTEB2) were shown to degrade pyrethroid (Deltamethrin), neonicotinoids (Thiamethoxam), and propargite. These bacterial isolates were identified using morphological, physiological, biochemical tests and molecular analysis.

The tolerance level of the bacterial isolates to different pesticide concentrations were done at preliminary level employing the Disc Diffusion Assay method (figure 4.8). The potential for these isolates to degrade pesticides was further investigated.

4.2.1.1 Disc diffusion assay

A passive disc diffusion experiment was conducted to indirectly quantify the toxicity of pesticides and analyse the susceptibility profiling of chosen bacterial isolates against their respective pesticides. Various concentrations (100 ppm to 300ppm) of pesticides were employed on the plane discs, and their zone of inhibition was quantified in millimeter. The results obtained from the passive disc diffusion experiment indicate that the isolate DRNB1 exhibited tolerance to deltamethrin concentration of up to 300 ppm, with no observable formation of zone of inhibition. The isolate EON2 also exhibited tolerance to ethion concentration of up to 300 ppm, with no observable formation of zone of inhibition. The isolate TXM1 exhibited a zone formation at the disc with thiamethoxam of concentration of 300ppm. A zone formation was observed in the plate streaked with the isolate SFN1 around the disc containing 300 ppm of spiromesifen. The isolate GLYB2 exhibited tolerance to 300 ppm of glyphosate without the formation of zone of inhibition. The isolate Q1T exhibited tolerance to quinalphos only at two concentrations of 100 ppm and 150 ppm with no zone formation. The isolate was susceptible to concentrations from 200 ppm to 300 ppm with an observable zone of inhibition. The isolate FXE1 exhibited tolerance to fenpyroximate concentrations up to 250 ppm and formed a zone of inhibition at the disc with 300 ppm concentration of fenpyroximate. The isolates PTEB2 and SFT1 exhibited tolerance to propargite and spiromesifen concentration up to 300 ppm respectively, with no observable zone of inhibition. The isolate F1T was susceptible to fenpyroximate at the concentration of 300 ppm with the formation of zone of inhibition. Passive disc diffusion assay was used by researchers to mainly study the antimicrobial properties of bacterial strains and to confirm the effect of chemical compounds (Singh *et al*., 2009). Badger *et al*., (2019) and Hooda *et al*., (2019) determined the antimicrobial susceptibility of *E.coli* and *Salmonella* strains through passive disc diffusion assay. Sharif and Mollick, (2013) studied the tolerance of gram-negative bacterium towards pesticide carbamate by disc diffusion assay. In the present study the pesticide tolerance of all the selected isolates were analysed using passive diffusion assay in a primary level.

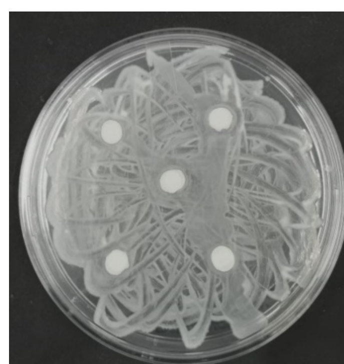
SFT1(100 ppm – 300ppm)

EON2(100ppm-300ppm)

F1T(100ppm-300ppm)

TXM1(100ppm-300ppm)

PTEB2(150ppm-300ppm)

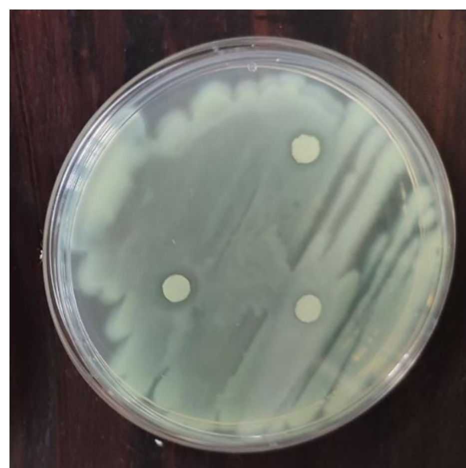


DRNB1(100ppm-300ppm)

GLYB2(150-250ppm)

GLYB2(300ppm)

SFN1(150-300ppm)



Q1T(200-300ppm)

FXE1 (100ppm)

FXE1(250-300ppm)

Figure No 4.8: Disc Diffusion Assay- Tolerance level of bacterial isolates to different concentration of pesticides

4.2.1.1 Characterization of bacterial isolates

4.2.1.1.1 Morphological Characterisation

- Colony Morphology and Gram staining

  The morphological characters of bacterial isolates were identified through microscopic observation. This showed that among the ten isolates, seven of them were gram negative, and the remaining three isolates were gram positive. The isolates DRNB1, EON2, SFN1, TXM1, FXE1, GLYB2, and PTEB2 were gram negative. The isolates F1T, Q1T, and SFT1 were gram positive. Figure 4.9

  The isolates DRNB1, EON2, SFN1, and TXM1 were gram negative rods, circular in shape, white to pale yellow, and with entire margins. F1T was gram positive rod, cream-coloured with irregular margins. The Q1T isolate was gram positive rod, with cream colony, and with irregular margins. The isolate FXE1 was gram negative rod, a bluish-green colour colony, and irregular margins. The isolate GLYB2 was gram negative rod, and colony colour cream. The isolate PTEB2 was gram negative rod, circular in shape, and yellow coloured colony. The SFT1 was gram positive rod, cream colour colony, and with a spread or jagged margin. The only isolate that displayed fluorescence features on Kings B agar plates was the FXE1.

  Pseudomonas isolates produced pigment on KBA under UV light, according to studies by Naik *et al*., (2008). *P. aeruginosa* produced fluorescent pigments that were either blue or yellow-green in color (Palumbo, 1972). The details of colony morphology, and cell characteristics of the tea garden soil bacterial isolates is mentioned in the Tables 4.15 and 4.16.

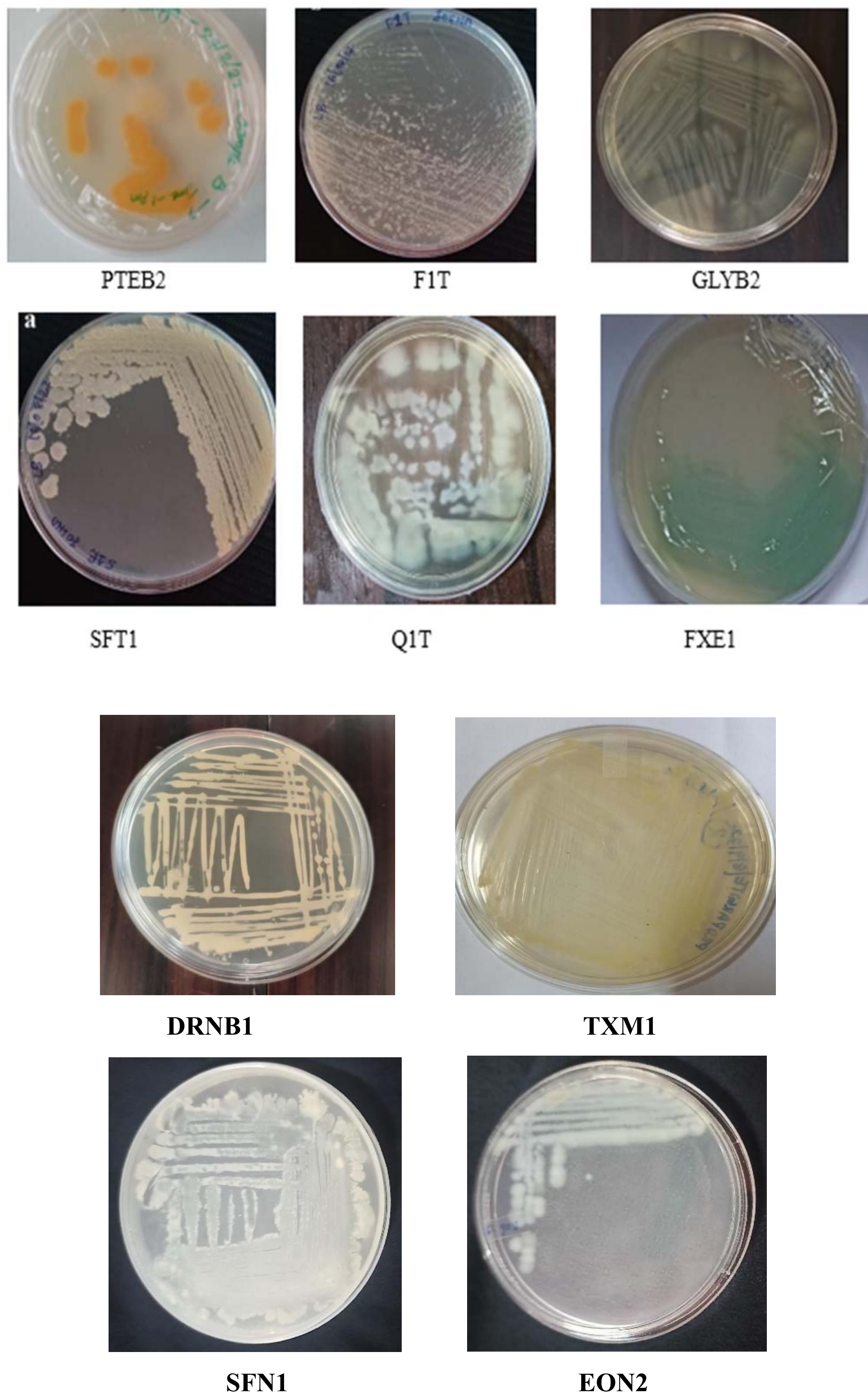


Figure No 4.9 : Colony Morphology of tea plantation soil bacterial isolates

Table 4.15: Colony morphology of Bacterial isolates DRNB1, EON2, SFN1, TXM2 and F1T. '+ve' indicates positive results and '-ve' indicates negative results.

Test Category	Test	DRNB1	EON2	SFN1	TXM1	F1T
Morphological characterisation	Gram Staining	-ve	-ve	-ve	-ve	+ve
	Colony Type	Circular shape, Light pink color	Circular shape, Light pink color	Circular shape, Light pink color	Circular shape, Light pink color	Colony with irregular margins, Cream color
	Flurosence Agar	-ve	-ve	-ve	-ve	-ve

Table 4.16: Colony morphology of Bacterial isolates Q1T, FXE1, GLYB2, PTEB2, and SFT1. . '+ve' indicates positive results and '-ve' indicates negative results

Test Category	Test	Q1T	FXE1	GLYB2	PTEB2	SFT1
Morphological characterisation	Gram Staining	+ve	-ve	-ve	-ve	+ve
	Colony Type	Colony with irregular margins, Cream color	Colony with irregular margins, bluish-green color	Circular shape, cream color	Circular shape, Light yellow color	Colony with spread or jagged margins, Cream color
	Flurosence Agar	-ve	+ve	-ve	-ve	-ve

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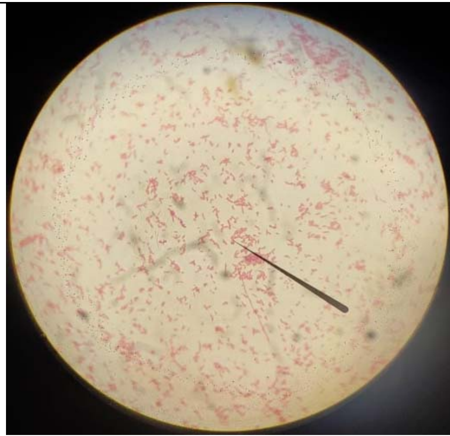
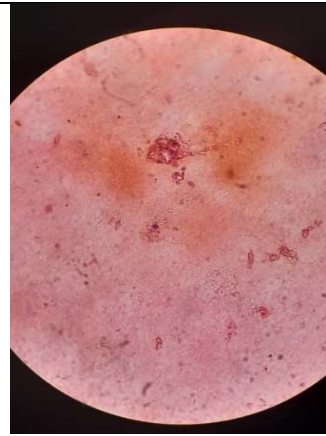
<tr><td>
EON2</td><td>
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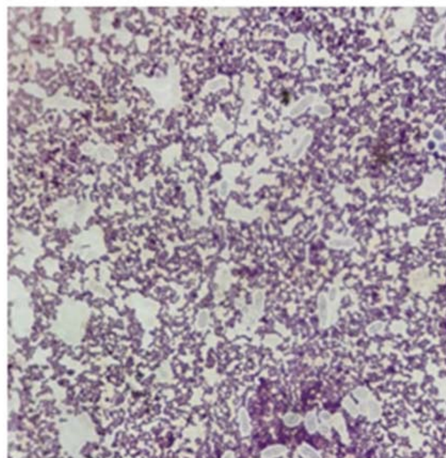
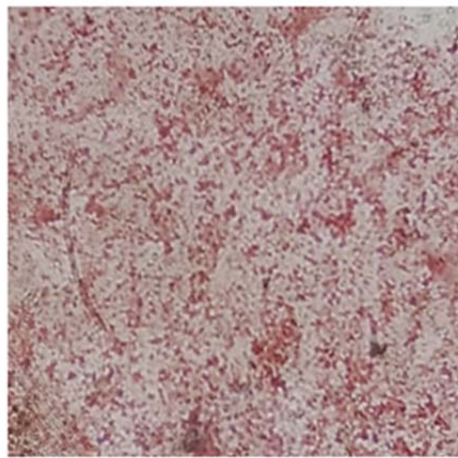
<tr><td>
Q1T</td><td>
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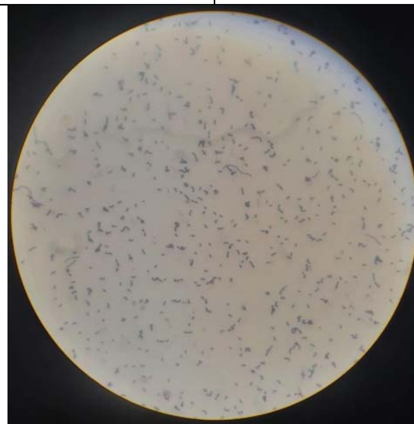
<tr><td colspan="2">
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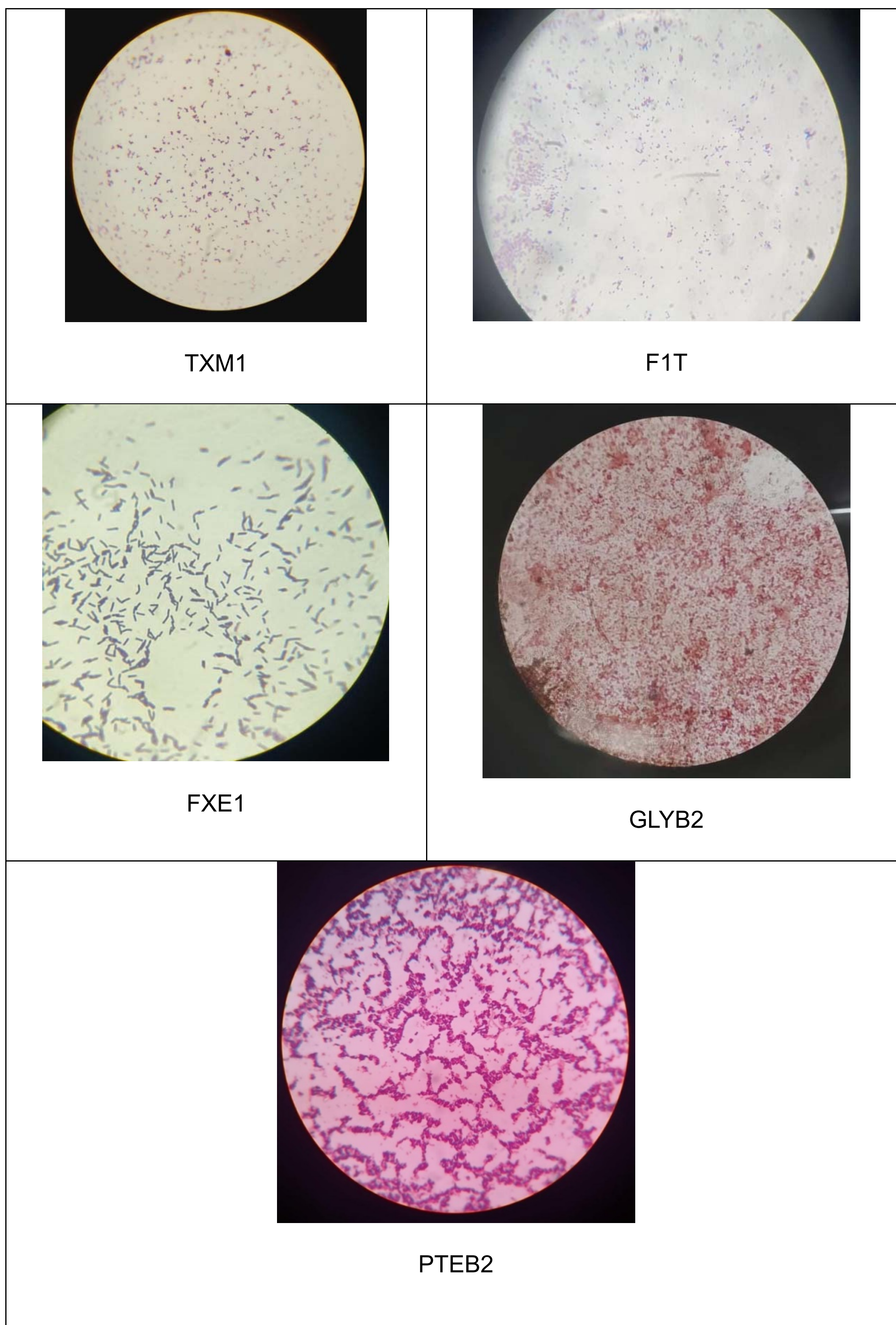


Figure No 4.10 Gram staining results of bacterial isolates

4.2.1.1.2 Biochemical Characterization

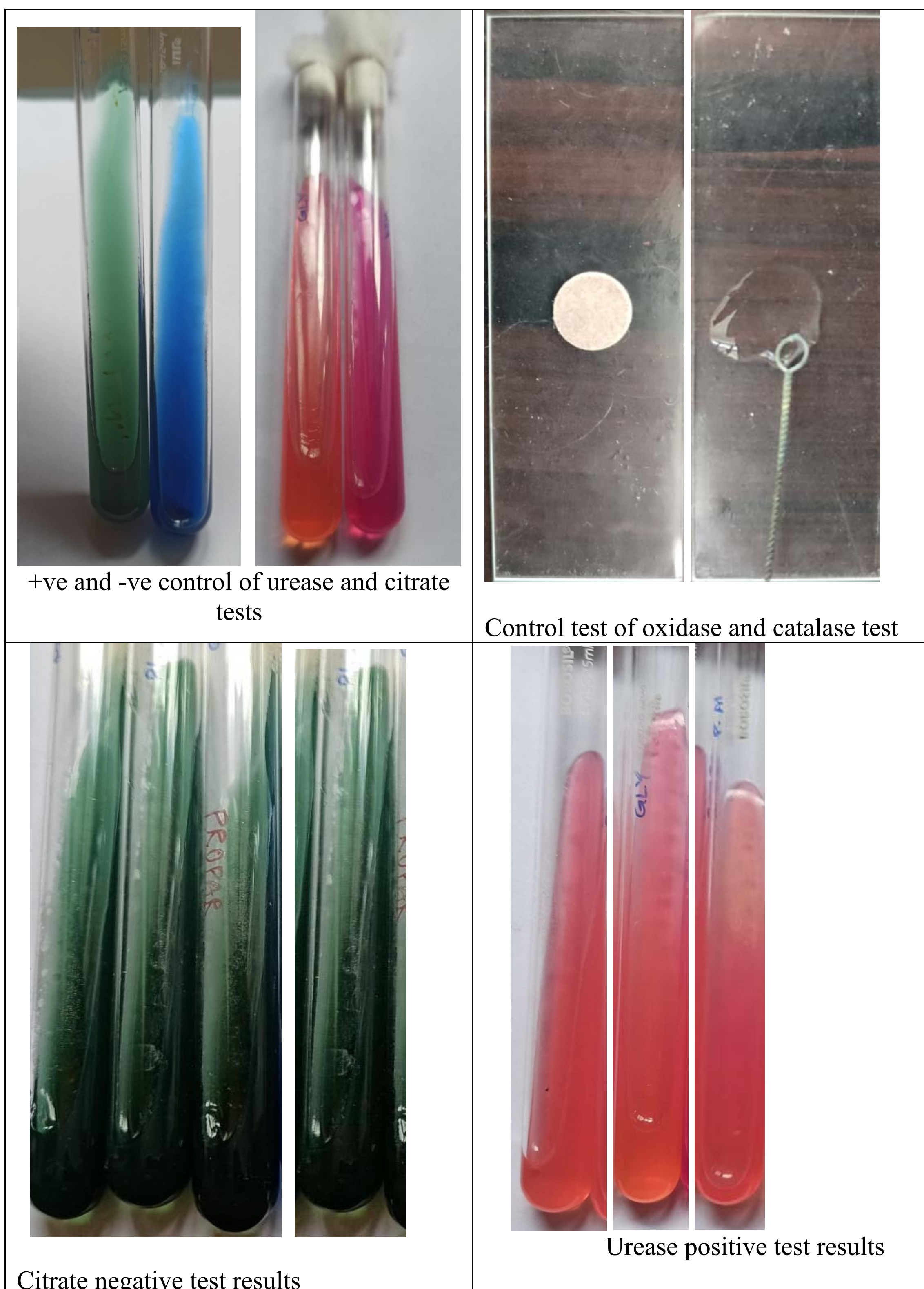
To characterize the bacterial isolates, various biochemical traits of each isolate were examined. The following are the biochemical tests performed: catalase, oxidase, citrate utilization, H_2S production, indole, methyl red (MR), and urease tests. The observed findings are presented in Table 4.17.

Biochemical characterization showed that all the isolates were catalase positive and negative for and Methyl red test and urease test. The isolates DRNB1, EON2, TXM1, and SFN1 were positive for H_2S production in the indole test and showed variable results for oxidase test. The isolates F1T and Q1T were negative for H_2S production, oxidase test, citrate utilisation test, and indole test. The isolate FXE1 was positive for the oxidase test, citrate utilisation test, and negative for the H_2S test and indole test. GLYB2 exhibited positive results for citrate utilisation test and negative for all other biochemical tests. The isolate PTEB2 was positive for the oxidase test and indole test. It exhibited negative results for citrate utilisation and H_2S production. The isolate SFT1 was positive for the citrate utilisation test and negative for all other biochemical tests carried out in the present study.

The genus of the bacterial isolates was approximately identified through biochemical characterization. Through biochemical characterisation, the isolates DRNB1, EON2, TXM1, and SFN1 were identified as it belongs to the genus *Stenotrophomonas.* The isolates F1T and Q1T belong to the genus *Paenibacillus*. The isolates GLYB2 and PTEB2 belong to the genus *Acinetobacter* and *Chryseobacterium* respectively. The isolates FXE1 and SFT1 belong to the genus *Pseudomonas* and *Bacillus* correspondingly.

Certain biochemical characteristics, such as gram-negative rod-shaped cells, a slight oxidase positive reaction, and a negative citrate test, are comparable to the criteria for the identification of the genus *Stenotrophomonas*. According to McMenamin et al., (2000) and Abbott and Peleg (2015), *Burkholderia* is often mistakenly confirmed as *Stenotrophomonas* based on biochemical features. Amoli *et al*., (2017) found that *Stenotrophomonas* is indole negative in nature. Djordjevic *et al*., (2000) reported the oxidase test positive for *Paenibacillus i*solates. Sharma *et al*., (2015) reported that *Paenibacillus* exhibits negative results for indole, citrate, and urease biochemical

tests. Hamdi *et al*., (2013) found that *Paenibacillus* isolates tested negative for catalase test.

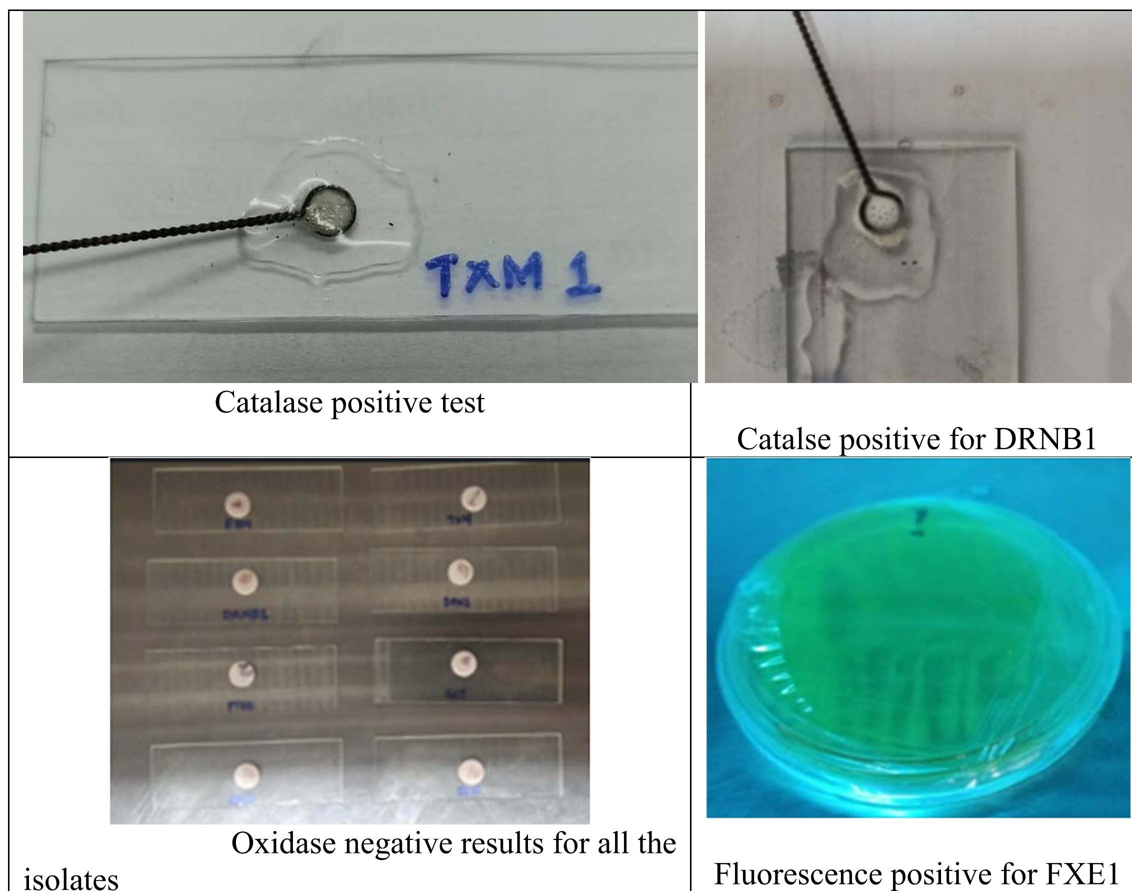


+ve and -ve control of urease and citrate tests

Control test of oxidase and catalase test

Citrate negative test results

Urease positive test results



Catalase positive test

Catalse positive for DRNB1

Oxidase negative results for all the isolates

Fluorescence positive for FXE1

Figure No 4.17: Biochemical tests done for the characterisation of tea plantation isolates

Bacterial isolates	Biochemical tests						
	Catalase	Oxidase	Citrate utilisation	Methyl Red test	Urease	H_2S production	Indole test
DRNB1	+ve	V	-ve	-ve	-ve	+ve	+ve
EON2	+ve	V	-ve	-ve	-ve	+ve	+ve
SFN1	+ve	V	-ve	-ve	-ve	+ve	+ve
TXM2	+ve	V	-ve	-ve	-ve	+ve	+ve
F1T	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Q1T	+ve	-ve	-ve	-ve	-ve	-ve	-ve
FXE1	+ve	+ve	+ve	-ve	-ve	-ve	-ve
GLYB2	+ve	-ve	+ve	-ve	-ve	-ve	-ve
PTEB2	+ve	+ve	-ve	-ve	-ve	-ve	+ve
SFT1	+ve	V	+ve	-ve	-ve	-ve	-ve

Table 4.17: Biochemical tests done fo the characterization of teaplantation soil bacterial isolates. '+ve' indicates a positive result. '-ve' indicates a negative result and 'V' indicates variable results.

The bacterial isolate *Acinetobacter* was verified by Raut *et al.,* (2020) using a variety of phenotypic tests, including catalase and citrate positive. A collection of non-motile catalase, oxidase, and indole-positive bacteria was identified as *Chryseobacterium* sp. by Christakis *et al*. in 2005. The yellow-colored pigmented colonies were identified as the *Chryseobacterium* strain by Tasic *et al*., (2012).

According to Segers *et al*., (1994), *Pseudomonas* species-specific characteristics include citrate utilization, catalase-positive, and oxidase-positive tests. A strain of *Bacillus sp*. that showed a positive reaction to the oxidase test and a negative reaction to the catalase, indole, and urease tests was obtained by Al.Dhabaan *et al*., (2019) and Awais *et al*., (2007). From all of these studies, it is inferred that bacterial isolates show different reactions for biochemical tests. So, through biochemical tests the genus and species of bacterial isolates cannot be confirmed accurately. Therefore, for precise confirmation of the identification of bacterial isolates molecular characterisation has to be done.

4.2.1.1.3 Phenotypic Characterization by Antibiotic susceptibility test

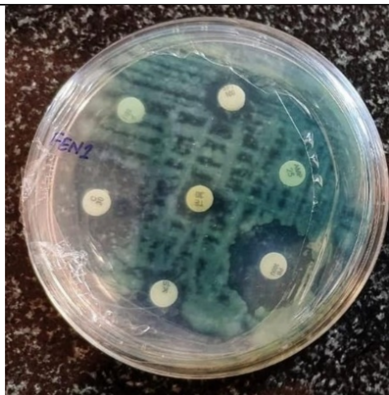
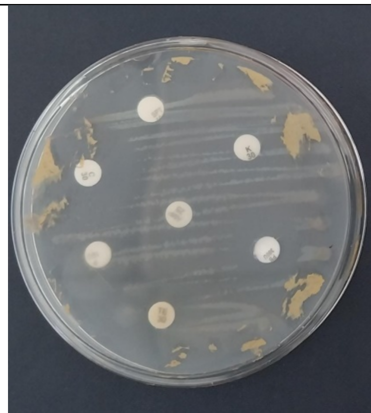
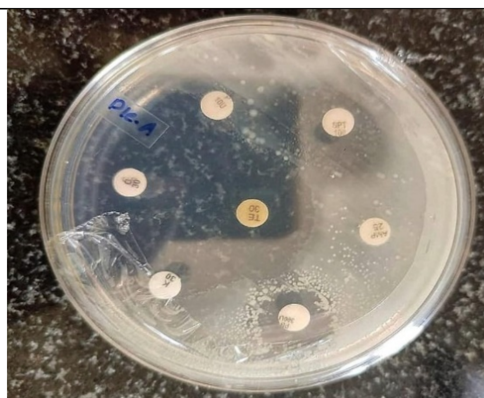
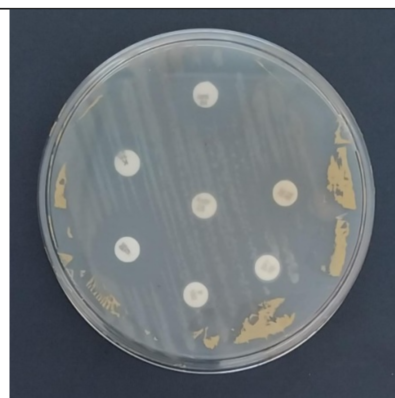
To characterize the phenotypic characteristics and confirm the pathogenicity of isolated bacterial strains, an antibiotic sensitivity test was performed. Seven different antibiotics (Chloramphenicol, Kanamycin, Spectinomycin, Polymyxin, Penicillin-G, Ampicillin, and Tetracycline) were used to characterize the phenotypes of bacterial strains. The specific response patterns of the test isolates were noted. The results were classified into two groups: resistant, which showed no zone of inhibition, and sensitive, which showed a zone of inhibition, based on the standard interpretation of diameter of zone of inhibition. The chart for zone diameter interpretive standard is given in Appendix.

Bacterial isolates DRNB1, SFN1, and EON2 showed total resistance towards chloramphenicol, spectinomycin, polymyxin, ampicillin, and tetracycline with no observable formation of zone of inhibition. The isolate DRNB1 and EON2 were sensitive to kanamycin (20mm) and resistant to Penicillin-G (23mm). The bacterial isolate SFN1 was resistant to Kanamycin (12mm) and Penicillin-G (20mm). The bacterial isolates F1T and Q1T showed resistance toward both Kanamycin and Ampicillin with no observable formation of zone of inhibition. Both these isolates showed sensitivity towards Chloramphenicol, Spectinomycin, and Tetracycline. These

isolates were resistant towards polymyxin (5mm) and penicillin G (14mm). The isolate TXM1 was resistant towards Kanamycin, Polymyxin, Spectinomycin, and Ampicillin and only exhibited sensitivity towards Chloramphenicol. The bacterial isolate GLYB2 showed sensitivity towards chloramphenicol, kanamycin, Polymyxin, and Tetracycline. This isolate was resistant to Spectinomycin, Penicillin G (18mm), and Ampicillin. The bacterial isolate PTEB2 exhibited resistance toward Spectinomycin and Polymyxin. PTEB2 showed moderate sensitivity towards kanamycin and tetracycline. The bacterial isolate FXE1 showed resistance towards Polymyxin and Ampicillin with no observable formation of zone of inhibition. The bacterial isolate SFT1 was completely resistant to spectinomycin, polymycin, penicillin G, ampicillin and tetracycline. The results are presented in Table 4.19 with the diameter of the zone of inhibition including the disc. The three isolates, DRNB1, SFN1, and EON2, displayed a similar comparable resistance and sensitivity patterns in the antibiotic susceptibility test. The antibiotics utilized in the study was ineffective against the isolate SFT1. The antibiotic susceptibility test aids in determining that which antibiotic will be the most successful against bacterial isolates (Reller, 2009). Additionally, it aids in phenotypic characterization of bacterial isolates.

Table 4.18: Antibiotic sensitivity and resistance shown by tea plantation soil isolates. The zone of inhibition is expressed in diameter(mm). C: Chloramphenicol, K: Kanamycin, SPC: Spectinomycin, PB: Polymyxin, P: Penicillin-G, AMP: Ampicillin, and TE: Tetracycline. R= Resistance. S= Susceptible. MoS/I = Moderately Susceptible/Intermediate.

Bacterial isolates	**Antibiotics (mcg/units)**						
	C^{30}	**K^{30}**	**SPC^{100}**	**PB^{300}**	**P^{10}**	**AMP^{25}**	**TE^{30}**
DRNB1	R	20mm S	R	R	23mm R	R	R
EON2	R	20mm S	R	R	23mm R	R	R
SFN1	R	12mm R	R	R	20mm R	R	R
TXM1	15mm	R	R	R	25mm R	R	10mm R
F1T	25mm S	R	12mm MoS	5mm R	14mm R	R	20mm S
Q1T	25mm S	R	12mm MoS	5mm R	14mm R	R	20mm S
GLYB2	23mm S	20mm S	R	10mm I	18mm R	R	20mm S
PTEB2	10mm R	15mm MoS	R	R	18mm R	R	15mm MoS
FXE1	11mm R	6mm R	10mm R	R	20mm R	R	11mm R
SFT1	8mm R	12mm R	R	R	R	R	R

 FEN1	 SFN1
 Q1T	 SFT1
FXE1	

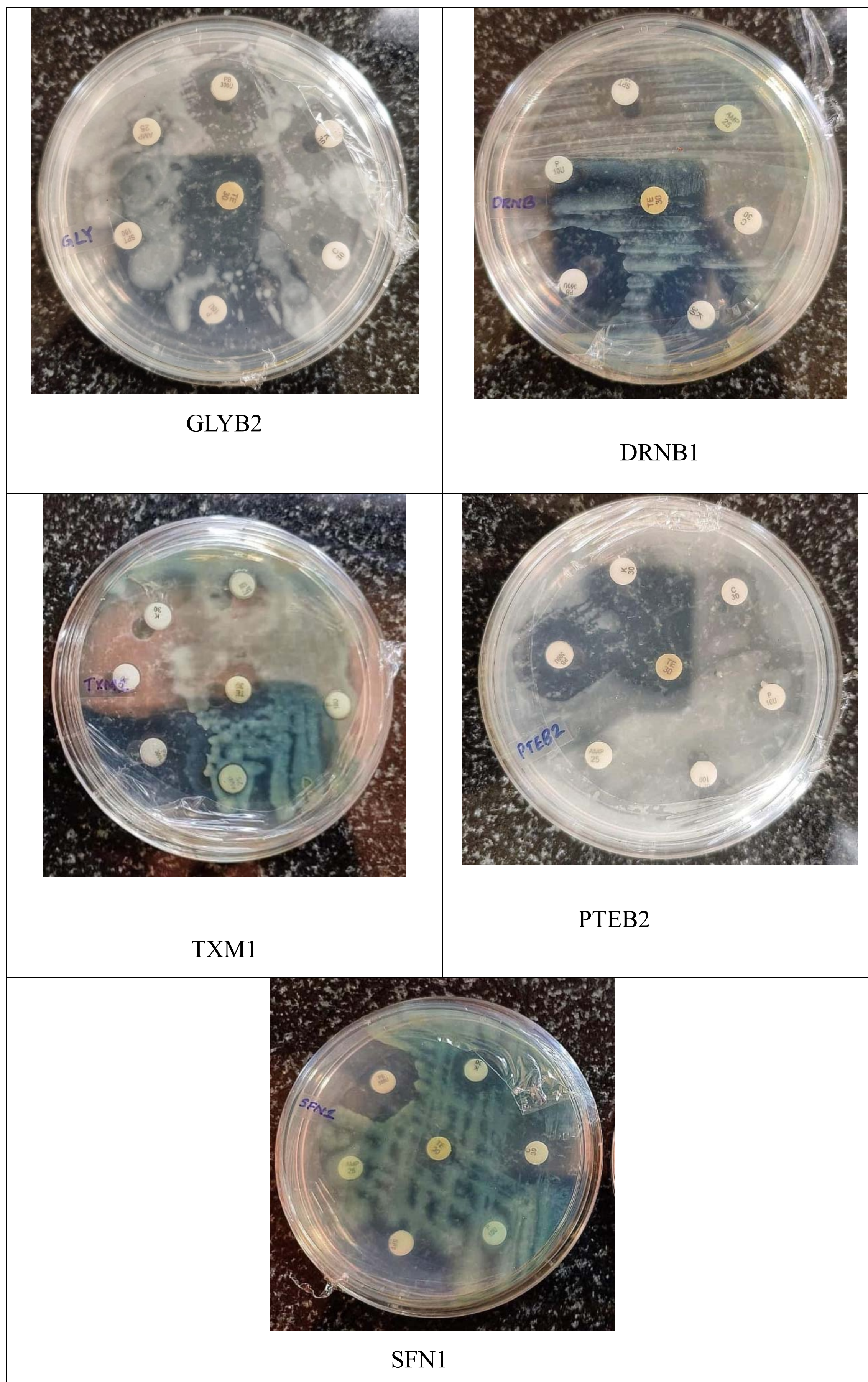


Figure No 4.12: Antibiotic susceptibility test of bacterial isolates showing zone of inhibition

4.2.1.1.4 Molecular Characterization and Phylogenetic tree construction

For the molecular characterization of bacterial isolates, the 16S rRNA partial sequencing method and phylogenetic analysis were used. This is one of the most sensitive and well-liked contemporary approaches used to identify the bacterial isolates. Strains from various genera were used to build the phylogenetic tree based on 16S rDNA sequences, which provided the purpose of identifying the phylogeny of the isolates. A maximum similarity index of >98% was used to identify the results of the 16S RNA partial gene sequencing of the tea garden bacterial isolates. Based on the similarity index, the isolates DRNB1, EON2, and TXM1 were identified as *Stenotrophomonas maltophilia strain DRNB1, Stenotrophomonas maltophilia strain EON2,* and *Stenotrophomonas maltophilia strain TXM2*. The isolate SFN1 was identified as *Stenotrophomonas geniculata strain SFN1*. The isolates F1T and Q1T were identified as *Paenibacillus alvei strain F1T and Paenibacillus alvei strain Q1T* respectively. The isolates GLYB2, PTEB2, SFT1, and FXE1 were identified as *Acinetobacter species GLYB2, Chryseobacterium cucumeris strain PTEB2*, *Bacillus subtilis strain SFT1* and *Pseudomonas aeruginosa* strain FXE1 respectively. Table 4.19

Stenotrophomonas species are a little bit smaller (0.7- 1.8 0.4-0.7 m), which differentiates them apart from the majority of other genus members. In terms of habitat and geography, *S. maltophilia* strains are reported to be widely dispersed in nature, and usually found attached to the roots of numerous plant species (Ryan et al., 2009). *S. maltophilia* has incredible potential for bioremediation as it deteriorates xenobiotics. *S. maltophilia* is known as a "Wonder bug" because of its enormous genetic and metabolic diversity. *Stenotrophomonas sp.* has been discovered to have a significant role in the biodegradation of many substances, including keratin (Yamamura *et al*., 2002), RDX (Binks *et al*., 1995), geosmin (Zhou et al., 2011), atrazine (Rousseaux et al., 2001), p-nitrophenol (Liu et al., 2007). It has been observed that *Stenotrophomonas maltophilia* is crucial for the bioremediation of chlorinated pesticides as chloropyrifos and endosulfan (Barragán-Huerta et al., 2007; Kumar et al., 2007). Dichlorodiphenyltrichloroethane (DDT) was broken down by soil isolates of *Stenotrophomonas* to l, l-dichloro-2, 2-bis (p-chlorophenyl) ethane(Mwangi et al., 2010). *S. maltophilia* has been found to have intrinsic resistance mechanisms against heavy metals as well as signaling or metabolic pathways.

Stenotrophomonas maltophilia R551-3 was isolated and described from Populus trichocarpa to enhance the growth and phytoremediation capability of poplar on marginal, contaminated soils (Nordberg *et al*., 2014). Several *Stenotrophomonas* strains have been shown to breakdown organic pollutants like pesticides [Y-j Shen et al.,2010], insecticides [H-Tang *et al*.,2012; Zhao et al.,2009], BTEX [Lee et al.,2002], PAH [Juhasz et al.,2002], and steroid hormones [Li et al.,2012].

In bioremediation and the agricultural sector, *Paenibacillus* species are crucial. Through bio flocculation or enzymatic processes, *Paenibacillus* species may be used in the removal or degradation of certain environmental contaminants. This species is utilized to get the removal of polyvinyl alcohol, heavy metals, and dyes (Ramya *et al*., 2008; Grady *et al*., 2016). *Paenibacillus alvei* was found to biodegrade fungicide, according to Birolli *et al*.,(2020) research. *A. baumannii* is a common bacterium in many natural settings with broad catabolic abilities. It has been shown to be very successful at breaking down diesel oil (Nkem et al., 2016), congo red (Li et al., 2015), and combinations of 1,4-dioxane and BTEX. Permethrin, malathion and Profenofos degradation by species from the *Acinetobacter* group has also been documented (Shan et al.,2009; Zhan et al., 2018; Kumar et al.,2021).

Pseudomonas bacteria, which are known to be metabolically active and capable of using a wide range of agrochemicals, were found in several soils that had been contaminated with pesticides, particularly organophosphate pesticides. In addition to degradation, the huge potential of pseudomonas species for plant growth development, bio-control, and nutrient mobilization has been thoroughly investigated (Yasouri,2006; Lakshmi *et al.,* 2008). The ability of *Pseudomonas* aeruginosa for the degradation of dimethoate (Deshpande *et al*.,2001), methyl parathion, and endosulfan (Senthilkumar *et al*.,2011), and quinalphos (Nair *et al.,*2015) is reported. There are no results about the degradation studies of fenpyroximate by *Pseudomonas* species. The biodegradation of pesticides is significantly facilitated by the species *Bacillus*. The effectiveness of *Bacillus* species in pesticide breakdown has been demonstrated in various research. The pesticides Chloprpyrifos (El-Helow *et al.,* 2013), endosulfan (Kumar *et al.,* 2014), and carbendazim (Salunkhe *et al.,* 2014) have all been documented to be degraded by bacillus species. The bacterium Chryseobacterium sp. can break down solid waste and pesticides. It breaks down pesticides such oxyfluorfen [Zhao *et al*., 2016], flubendiamide [Jadhav, 2016,] organochlorine pesticides [Qu J *et*

al. 2015], and carbendazim [Silambarasan, 2020]. The variety of bacterial species and patterns of pesticide degradation may be unrelated to the geographic and ecological origins of the isolates. (Figure 4.13 to Figure 4.21).

Table 4.19: Identification of bacterial isolates based on molecular properties.

Bacterial isolate	**GenBank Accession No**	**Molecular identification**	**Closest relative**	**Similarity (%)**
DRNB1	ON384040	*Stenotrophomonas maltophilia*	*Stenotrophomonas*	99.59
EON2	OP122562	*Stenotrophomonas maltophilia*	*Stenotrophomonas*	97.8
SFN1	ON384044	*Stenotrophomonas geniculata*	*Stenotrophomonas*	97.99
TXM1	OQ361827	*Stenotrophomonas maltophilia*	*Stenotrophomonas*	99.15
F1T	OQ361799	*Paenibacillus alvei*	*Paenibacillus Bacillus*	99
Q1T	OQ361777	*Paenibacillus alvei*	*Paenibacillus Bacillus*	98.87
GLYB2	ON384043	*Acinetobacter sp.*	*Acinetobacter baumannii*	100
PTEB2	OQ361849	*Chryseobacterium cucumeris*	*Chryseobacterium indologens*	98.25
FXE1	ON384042	*Pseudomonas aeruginosa*	*Pseudomonas*	99.63
SFT1	OQ361800	*Bacillus subtilis*	*Bacillus*	97.8

The bacterial isolates DRNB1, EON2, and TXM1 were identified as the bacterial genuc *Stenotrophomonas maltophilia.* They are isolated from soil samples contaminated with different pesticides from different sampling sites. The bacterial isolates F1T and Q1T were, *Paenibacillus alvei* that degrades fenpyroximate and quinalphos. These isolates were isolated from the transition area sampling site (S4). The distribution of bacterial species in the sampling sites is dependent on agricultural practices and the use of pesticides over that site.

- Phylogenetic position and trees of isolated bacterial strains

Phylogenetic trees were created by analysing the sequences that had the highest similarity to the consensus sequences of the isolates recorded in the NCBI GenBank database using BLAST analysis. A minimum number (8 to 10) of GenBank submitted data sets were taken to generate a phylogenetic tree for accurately estimating the

phylogenetic position of isolated strain. The fig. 4.19 to 4.28 represents the phylogenetic position of all bacterial strains isolated in the present study.

The isolate with sample code DRNB1 was identified and submitted to GenBank as a new strain of *Stenotrophomonas maltophilia* DRNB1 that showed 99.59% sequence homology with *Stenotrophomonas maltophilia* and other strains. The isolate EON2 was proposed as *Stenotrophomonas maltophilia* EON2 and it showed 97.8% similarity to *Stenotrophomonas maltophilia* CF13 and other related species. Isolate SFN1 was identified as *Stenotrophomonas geniculata* SFN1 which showed 97.99% similarity to *Stenotrophomonas*. The isolate TXM1 was identified and submitted to GenBank as a new strain of *Stenotrophomonas maltophilia* TXM2 that showed 99.15% sequence homology with *Stenotrophomonas maltophilia* and other strains.

The isolates F1T and Q1T were submitted as *Paenibacillus alvei* F1T and *Paenibacillus alvei* Q1T respectively. In addition, F1T showed 99% similarity with *Paenibacillus bacillus* HPB2 and Q1T (98.87%) homology with *Paenibacillus bacillus* TSIII-14. The isolate GLYB2 was identified as *Acinetobacter baumannii*. GLYB2 and it was submitted to the GenBank as it showed 100% similarity with *Acinetobacter viviannii* CPOC48. The isolate PTEB2 was proposed as *Chryseobacterium cucumeris* PTEB2 and it showed 98.25% similarity to *Chryseobacterium indologens* MP25. The isolate FXE1 was identified as *Pseudomonas aeruginosa* FXE1 which showed 99.63% similarity to *Pseudomonas* 22112. The isolate SFT1 was identified and submitted to GenBank as a new strain of *Bacillus subtillis* SFT1 that showed 97.8% sequence homology with *Bacillus AJ4* and other strains.

Phylogenetic inference was used to find PCR product sequence similarity hits for taxonomy identification.The neighbour joining approach was used to build a phylogenetic tree. Figure 4.13 to 4.21 illustrates the phylogenetic tree.

Figure 4.13: Phylogenetic tree of propargite degrading isolate *C.cucumeris* PTEB2 species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11.

Figure 4.14: Phylogenetic tree of propargite degrading isolate *S.geniculata SFN1* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11.

Figure 4.15: Phylogenetic tree of deltamethrin degrading isolate *S. maltophilia DRNB1* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11.

Figure 4.16: Phylogenetic tree of ethion degrading isolate *S. maltophilia EON2* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11.

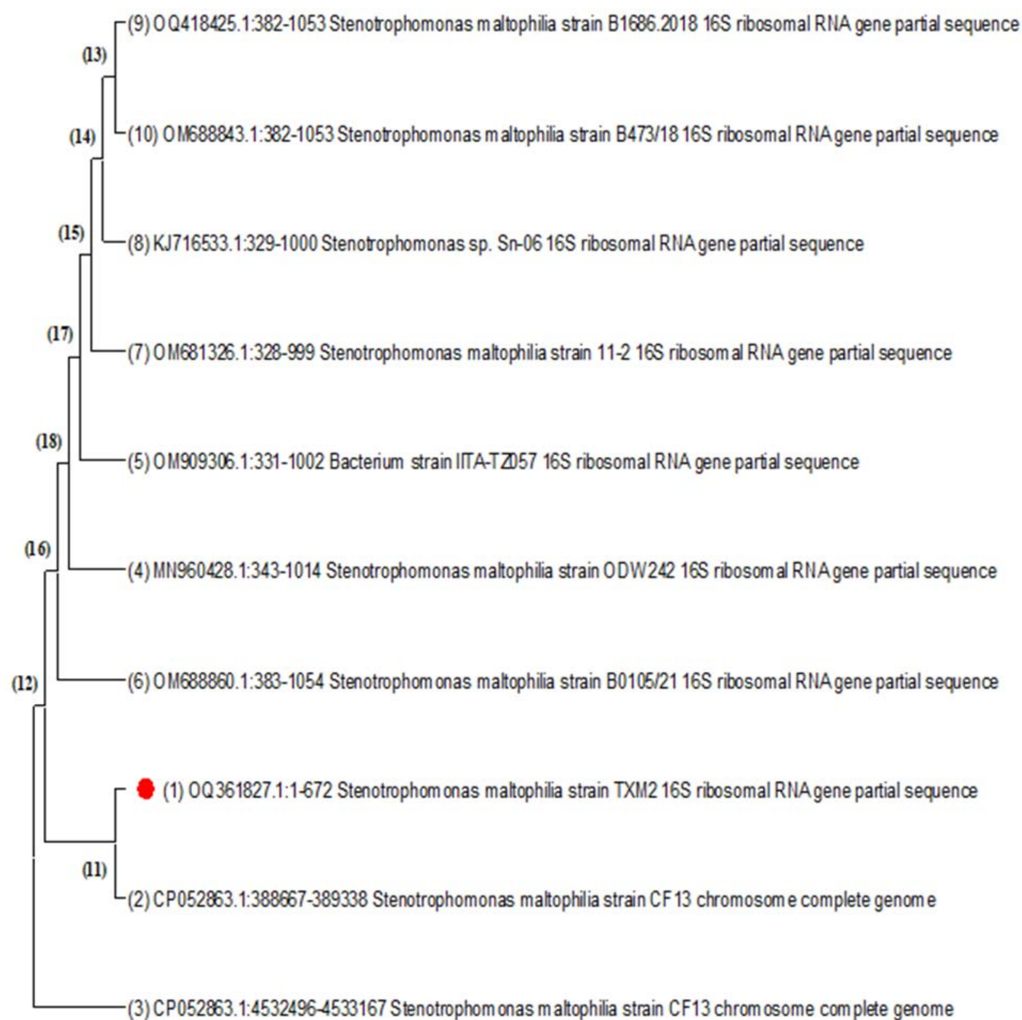


Figure 4.17: Phylogenetic tree of thiamethoxam degrading isolate *S. maltophilia TXM2* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11.

Figure 4.18: Phylogenetic tree of fenpyroximate degrading isolate *P. alvei F1T* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11.

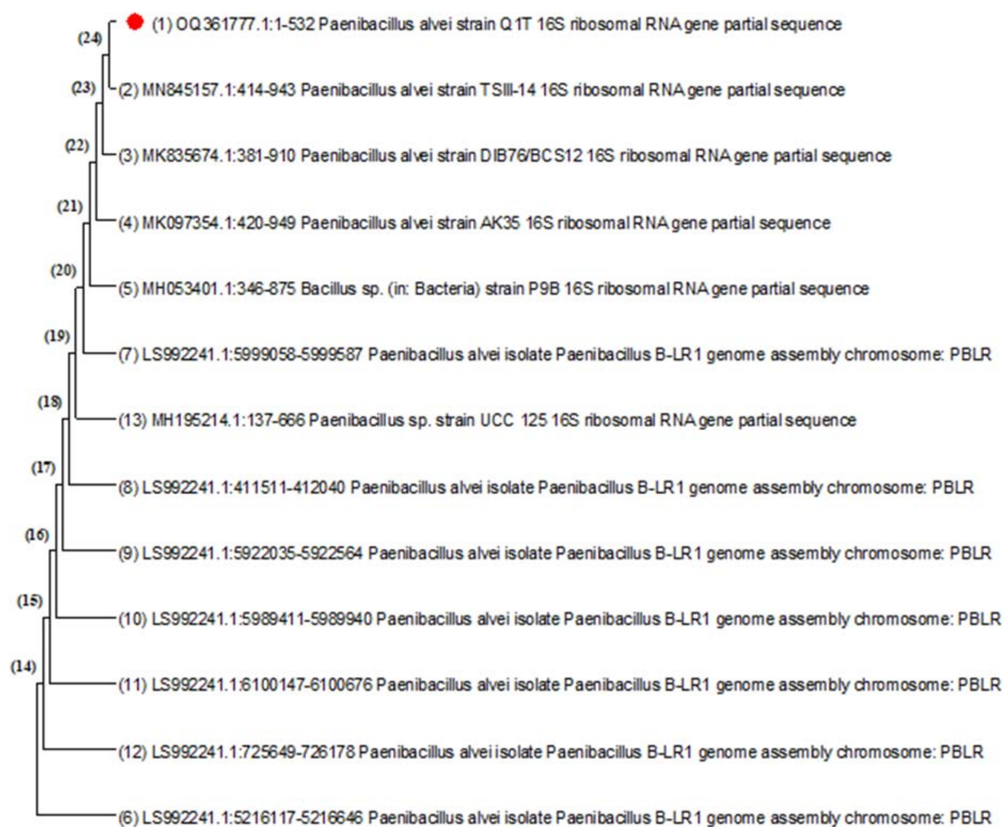


Figure 4.19: Phylogenetic tree of quinalphos degrading isolate *P. alvei Q1T* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11

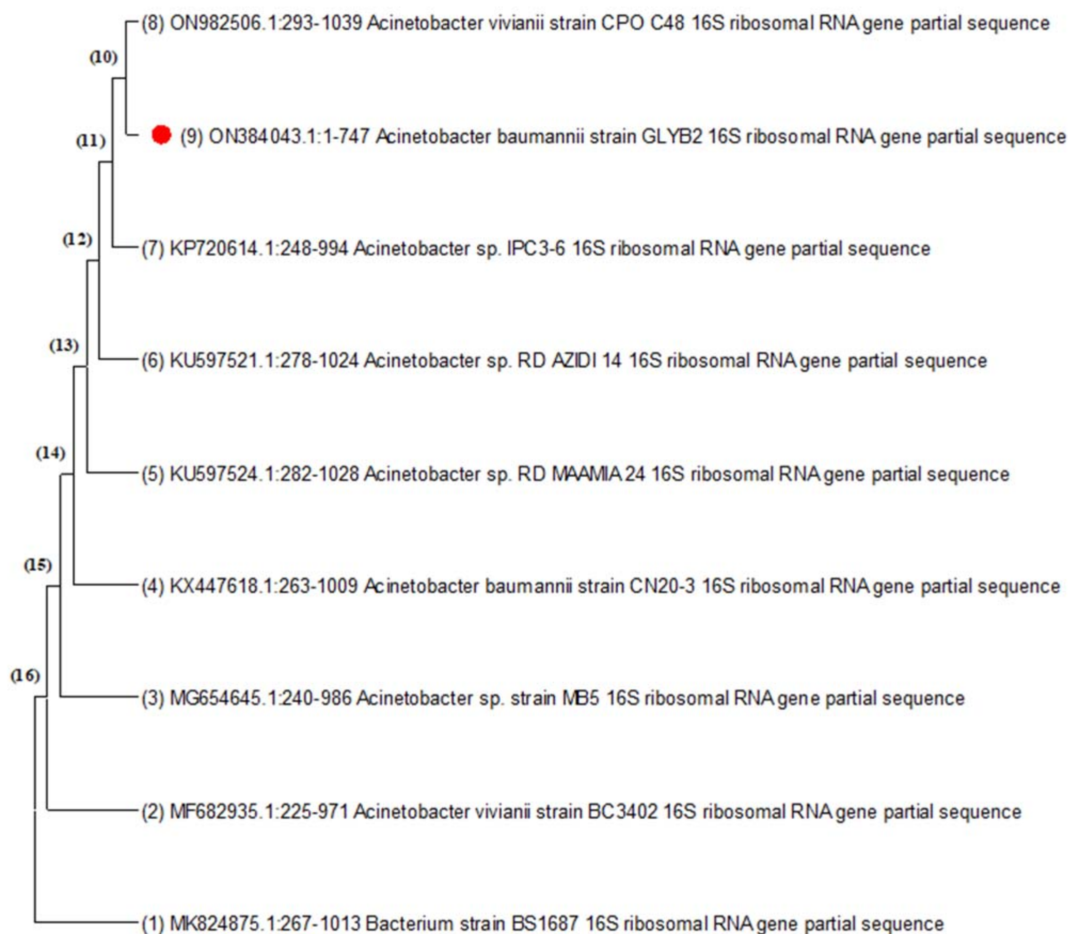


Figure 4.20: Phylogenetic tree of glyphosate degrading isolate *A. baumannii GLYB2* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11

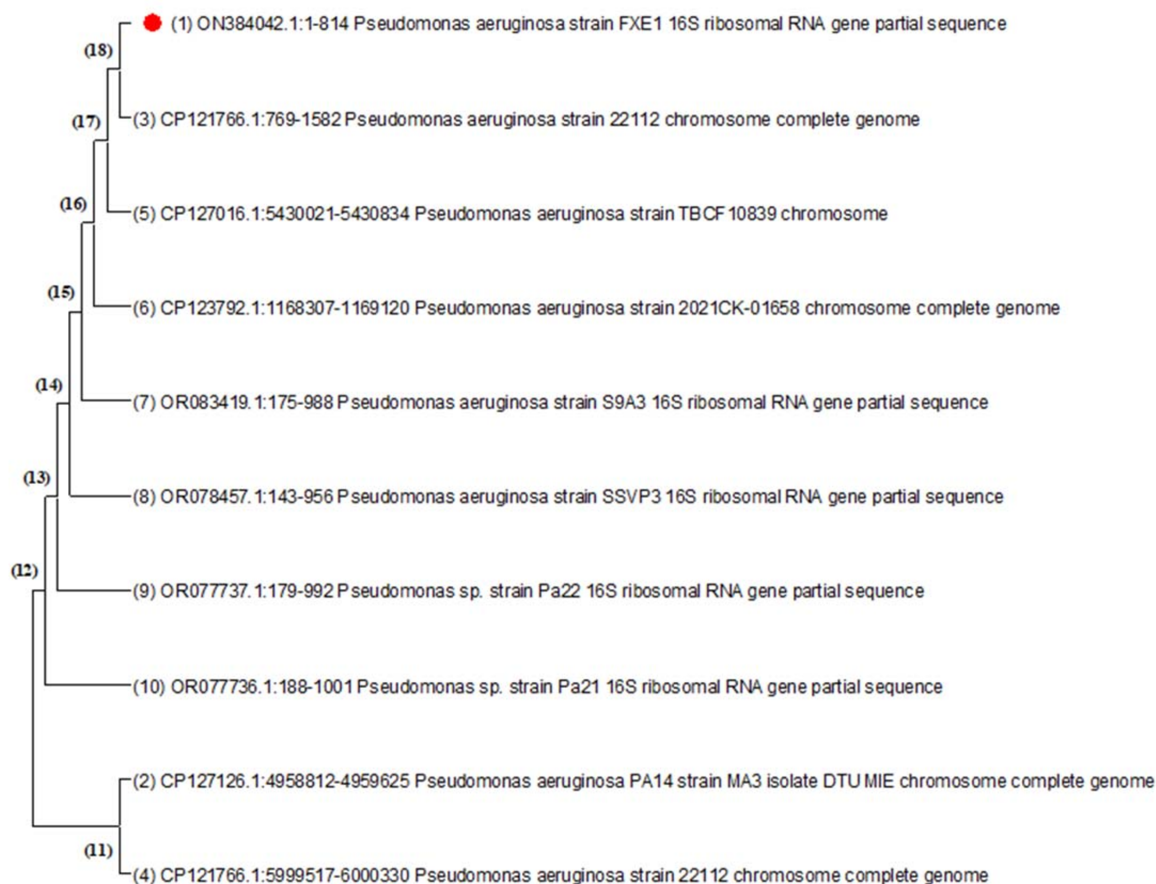


Figure 4.21: Phylogenetic tree of fenpyroximate degrading isolate *P. aeruginosa FXE1* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11

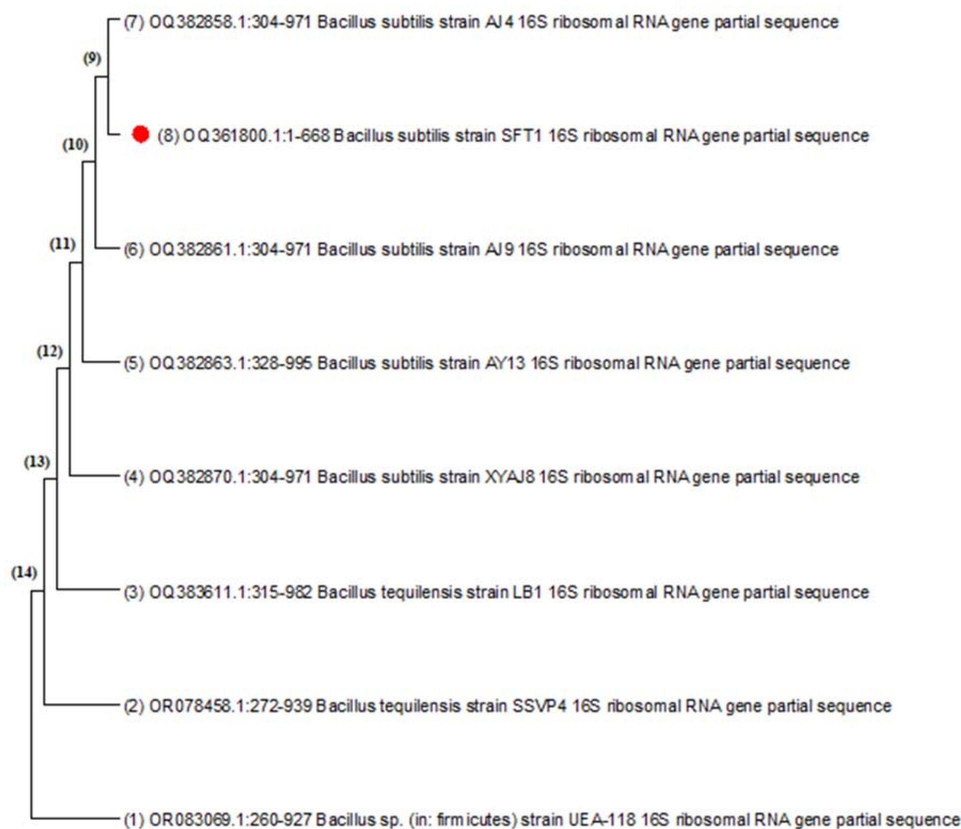


Figure 4.22: Phylogenetic tree of spiromesifen degrading isolate *B. subtilis SFT1* species by neighbour joining method. The bootstrap consensus tree inferred from 100 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA11

4.2.2 Biosurfactant and Biofilm Production of bacterial isolates

4.2.2.1 Biosurfactant Production

Biosurfactant or surface-active substances are a diverse set of surface-active molecules produced by microorganisms that either attach to cell surfaces or are ejected extracellularly in the growth medium (Fietcher, 1992). Biosurfactants are gaining popularity because of their uses in petrochemical production and environmental protection. Microbial surfactants are categorized based on their biochemical properties. Peptides, fatty acids, phospholipids, glycolipids, antibiotics, lipopeptides, neutral lipids, fatty acids, and Polymeric Microbial Surfactants (PMS) are all examples of biosurfactants (Shafi and Khanna, 1995; Lin, 1996).

According to Foght *et al*., (1989), Francy *et al.,* (1991), and Marchesi *et al*., (1991), the environmental uses of biosurfactants are largely associated with the bioremediation of petroleum hydrocarbons in groundwater and soil as well as the

degradation of hazardous chemicals. The application of microbial biosurfactants in environmental protection includes improving oil recovery, containing oil spills, biodegrading, and detoxifying soils and industrial effluents that have been contaminated with oil. Rhamnolipids are among the most well-researched microbial surfactants and are a member of the glycolipids group of biosurfactants. Glycolipids, which comprise rhamnolipids, trehalose lipids, and soporose lipids, are the most common kind of biosurfactant. Glycolipid biosurfactants are often carbohydrates combined with long-chain aliphatic acids or hydroxy aliphatic acids.

This study examined the production of biosurfactant rhamnolipids by native tea plantation bacterial isolates. According to the testing technique used, the development of a dark blue zone surrounding the bacterial colony after five days of incubation indicated a favourable response to produce rhamnolipids. The study inferred that among ten native tea plantation bacterial strains, DRNB1, EON2, TXM2 and SFN1(*Stenotrophomonas maltophilia and S. geniculate*) indicated the strongest surfactant production followed by GLYB2 (*Acinetobacter*), FXE1 (*Pseudomonas aeruginosa),* SFT1 (*Bacillus subtillis*) and PTEB2 (*Chryseobacterium cucumeris*). The strain PTEB2 showed the least biosurfactant production having developed a low amount of blue zone following incubation of the plate for more than five days. The study concludes that all the isolated bacterial strains have the potential for biosurfactant production that enhances the degradation of pesticides in the environment. SDS was taken as the positive control. Table 4.20 consists the result of biosurfactant assay.

Researchers have described the biosurfactant producing ability of various microorganisms. CTAB-methylene blue indicator plates, and FT-IR spectrum analysis were used to analyse the biosurfactant producing ability of *Stenotrophomonas maltophilia* JJC-1, *Enterococcus faecalis* JJC-2, and *Pseudomonas fluoroscens* JJC-3 (Thaniyavarm, 2003). Kim *et al*., (1997) found that the surface tension reducing action of *Bacillus subtillis* C9 was stable over the range of pH 5 to 9.5. Thaniyavarn *et al.,* (2003) described a lipopeptide-type biosurfactant generated by *Bacillus licheniformis*.

The biosurfactant production ability of *S. maltophilia* has been studied by several researchers. Deepthi et al., (2014) identified *Stenotrophomonas maltophilia* with the

capacity to produce rhamnolipid biosurfactant. Hemlata *et al*., (2015) identified an iron-chelating biosurfactant from *S. maltophilia*, and they found that the production of the biosurfactant was higher at pH 7 and 37 °C. Singh et al., (2015) isolated *S. maltophilia* BR-12, from an oil refinery in Vadodara with the ability to produce biosurfactant. They also reported that the degradation of oil (pyrene) was enhanced by the biosurfactant produced. Gargouri *et al*., (2017) isolated biosurfactant producing *S. maltophilia* that are effective in the breakdown of diesel and used motor oil as well as the solubilization of phenanthrene. Larik *et al.,* (2019) isolated the biosurfactant-producing *S. maltophilia* 5DMD strain that degrades two petrochemical hydrocarbons.

Several studies suggest the production of rhamnolipid biosurfactants by *P.aeruginosa*. (Lang and Wullbrandt, 1999). Gunther *et al*., (2005), for example, isolated biosurfactant producing strain *P. chlororaphis*. Tuleva *et al.,*(2002) discovered soil bacterium *P. putida* capable of producing rhamnolipids which uses hexadecane as a carbon source. Five bacterial strains were identified by Tripathi *et al*. in 2020. They also examined the ability of the biosurfactant production of all the selected strains. They discovered that *S. maltophilia* IITR47 produced more biosurfactant than *Pseudomonas aeruginosa* IITR48 among the five bacterial strains. Several experts have discovered *Pseudomonas* species that produce biosurfactants, including *P.aeruginosa* MR01 from oil excavation sites in the south of Iran (Lotfabad *et al*., 2009), *P.aeruginosa* WJ-1 from China (Xia *et al*., 2012), and glycolipid biosurfactant producing *P.aeruginosa* HAK01 (Khademolhosseini *et al*., 2019).

Table 4.20: Biosurfactant (rhamnolipid) production by tea plantation soil isolates

STRAINS	BIOSURFACTANT PRODUCTION (CTAB)
DRNB1	Strong positive
EON2	Strong positive
SFN1	Strong positive
TXM2	Strong positive
FXE1	Strong positive
GLYB2	Strong positive
PTEB2	Negative
SFT1	Positive
F1T	Weak positive
Q1T	Weak positive

Multiple studies have shown that the bacterial species *Paenibacillus alvei* and *Acinetobacter baumannii* are capable of synthesizing biosurfactants. *Paenibacillus alvei*, an indigenous bacterial strain with the capacity to produce biosurfactants, was identified by Najafi *et al.*, in 2010. *Paenibacillus alvei* were isolated from crude oil by Gudina *et al.*, (2015), who additionally examined the capacity of the organism for aerobic and anaerobic degradation at 40°C. *Acinetobacter baumannii* MN3 for the breakdown of crude oil was isolated by Parthipan *et al.* in 2017. The study also revealed that pH 7 and 40 °C are the ideal parameters for the production of biosurfactants. Gupta *et al.,* 2020, identified *Acinetobacter species* BJ5 that produces glycolipid biosurfactants for the breakdown of pyrene. *Acinetobacter baumannii* OCB1 which produces lipopeptide biosurfactants aided in the breakdown of petroleum crude oil (Goveas and Sajankila, 2020). Saimmai *et al.*,(2012) discovered a biosurfactant-producing microbial consortia SC-9 including the *Chryseobacterium* species from oil-contaminated sites. The observations of these recent researchers on biosurfactant production by bacteria validate the findings of the present study that biosurfactant enhances the degradation ability of bacteria. Very limited information was available on the production of biosurfactants by *Chryseobacterium* species. There is a need for further studies to determine the biosurfactant production of *Chryseobacterium cucumeris.*

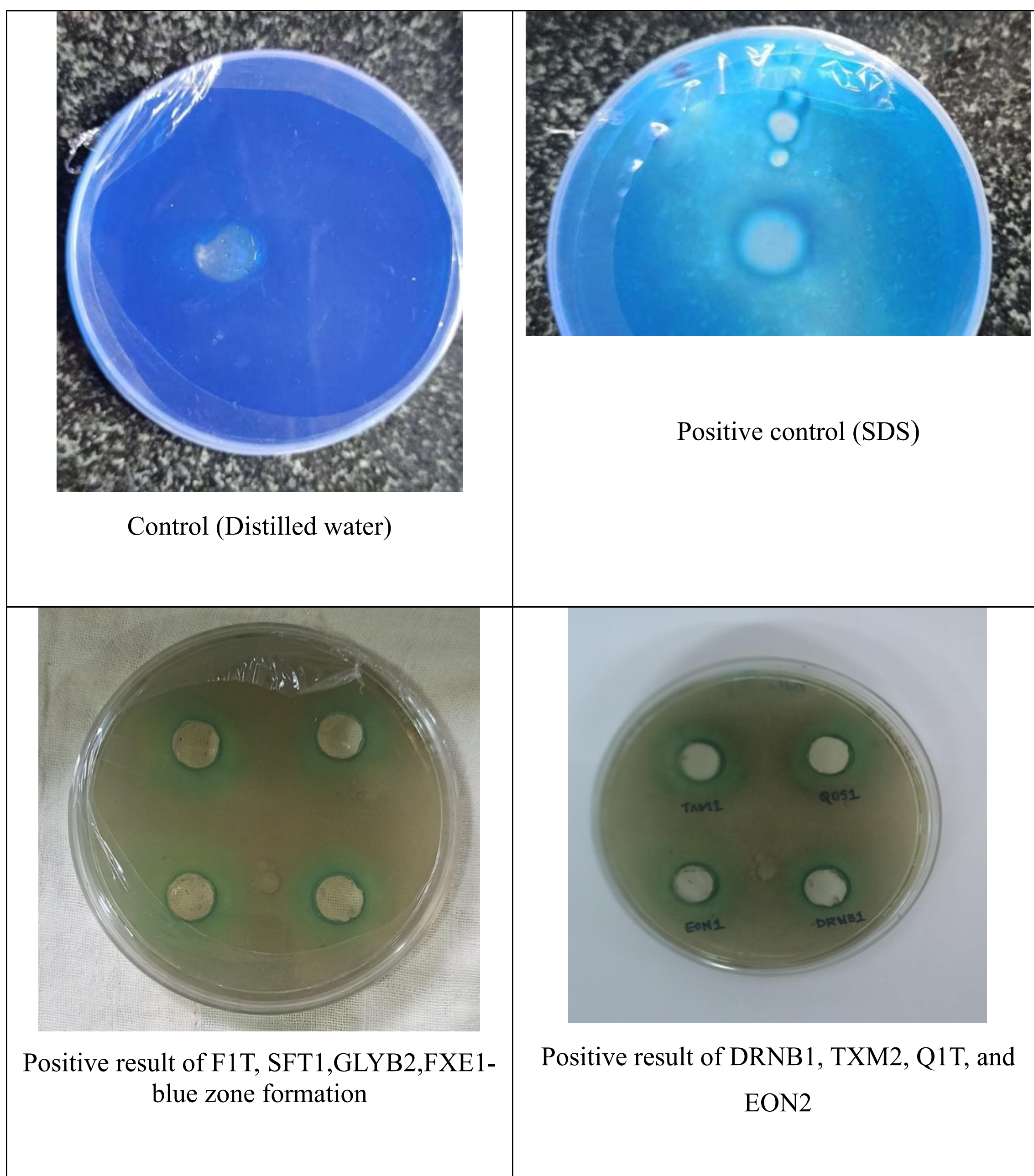


Figure 4.23 : Biosurfactant production of tea plantation bacterial isolates.

4.2.2.2 Biofilm Production

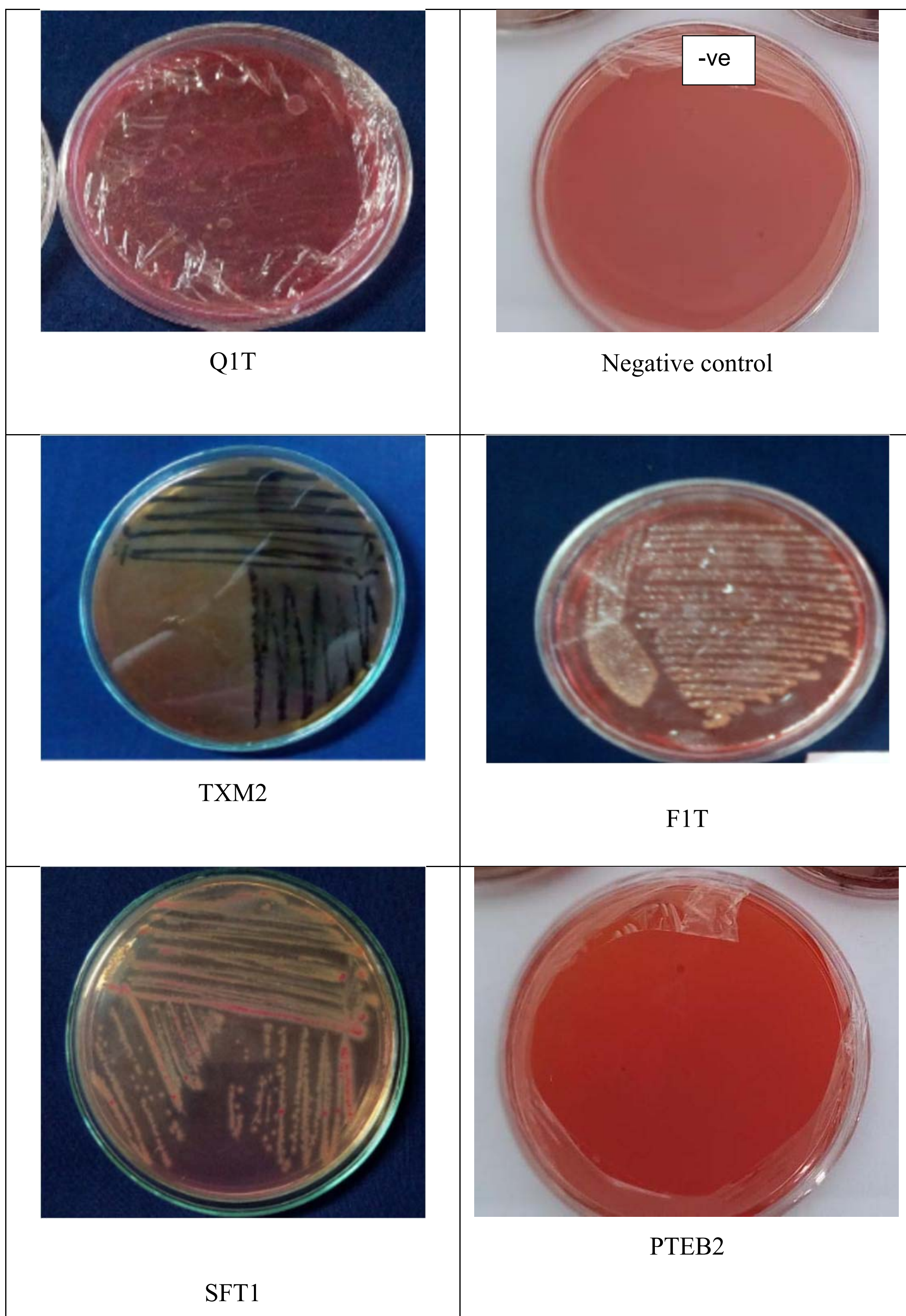
Bacterial biofilms are colonies of bacteria that have formed a self-made matrix and are affixed to a surface or one another. Proteins (such as fibrin), polysaccharides (such as alginate), and eDNA make up the biofilm matrix. Microbes can adapt to a stressful environment by creating a protective matrix with the aid of biofilm. Due to its broad flexibility, abundance of biomass, and superior ability to absorb, immobilize, or decompose toxins, biofilm-mediated bioremediation is a promising method for the removal of environmental pollutants (Mishra *et al.*,2022). In this investigation, two methods were used to determine the biofilm production of bacterial isolates: the tube method and the Congo red Agar media method. Tea plantation bacterial isolates were examined to produce a red biofilm around the test tube, which can be observed after being stained with safranin. The formation of dark black coloured colonies in the Congo red agar media indicates the ability to form biofilm.

The isolates DRNB1, EON2, SFN1, TXM2, FXE1, and GLYB2 had the strongest capacity to create biofilms, followed by SFT1, F1T, and Q1T. The Congo red agar media colonies of PTEB2 were colourless and had a very low chance of producing biofilms. DRNB1, EON2, SFN1, TXM2, FXE1, and GLYB2 produced dark, black, and red colonies on the Congo red agar media. In the Tube method, DRNB1, EON2, SFN1, TXM2, FXE1, and GLYB2 formed red biofilm on the walls of the test tube when stained with safranin. A weak biofilm formation was observed in the walls of the test tube inoculated with SFT1, F1T, and Q1T. No safranin-stained red coloured biofilm was observed in the test tube inoculated with PTEB2. The results are depicted in Table 4.21

Table 4.21: Biofilm formation by tea garden soil isolates

STRAINS	**BIOFILM FORMATION**	
	Tube method	**Congo red media**
DRNB1	Red biofilm	Dark black colonies
EON2	Red biofilm	Dark black colonies
SFN1	Red biofilm	Dark black colonies
TXM1	Red biofilm	Dark black colonies
FXE1	Red biofilm	Dark black colonies
GLYB2	Red biofilm	Dark black colonies
PTEB2	No biofilm	Colourless colonies
SFT1	Weak biofilm formation	Light red colonies
F1T	Weak biofilm formation	Light red colonies
Q1T	Weak biofilm formation	Light red colonies

Figure 4.24: Biofilm formation in Congo red agar media



Q1T

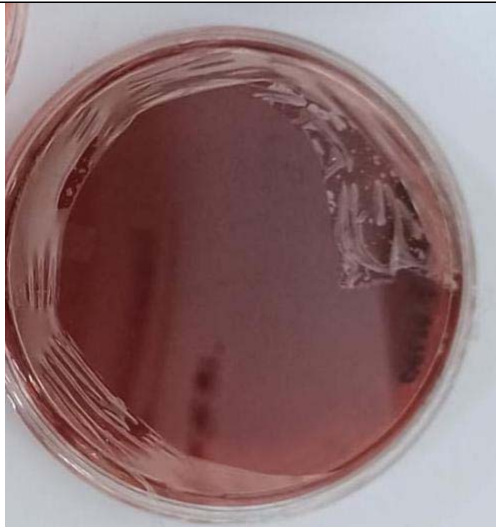
Negative control

TXM2

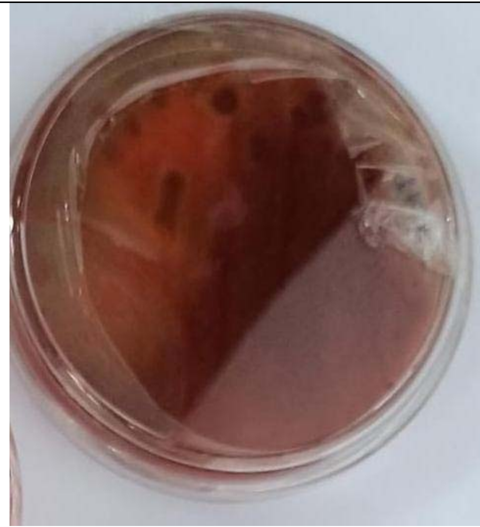
F1T

SFT1

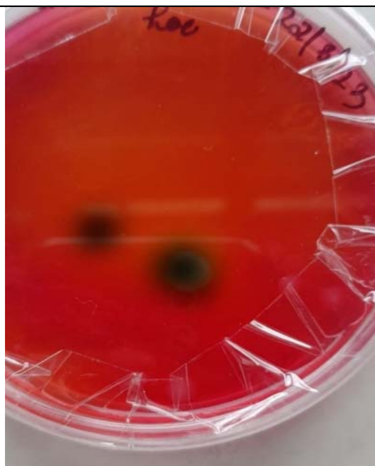
PTEB2



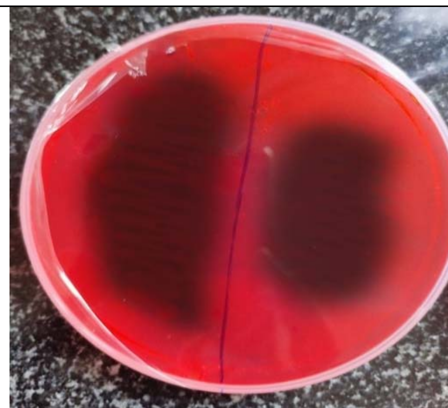
DRNB1



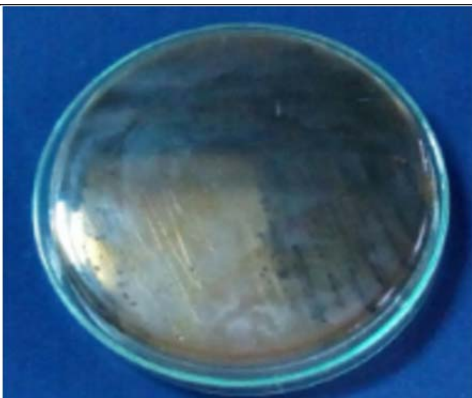
EON2



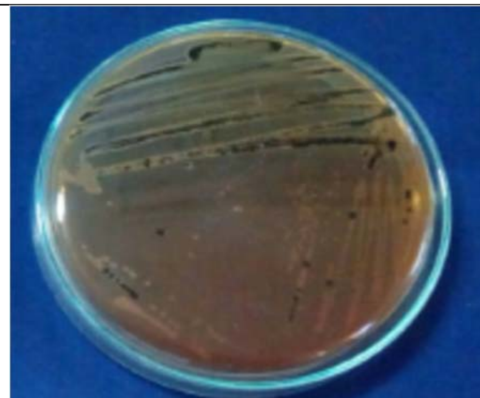
SFN1



Positive control



GLYB2



FXE1

Biofilms speed up the breakdown of xenobiotic substances (Deksissa and Vanrolleghem, 2003). According to several researchers, S. *maltophilia* is highly resistant to antibiotics because of its great capacity to form biofilm on moist surfaces (Brooke, 2012; Wu *et al.*, 2021). In a biofilm reactor, the biofilm development of *S. maltophilia* increases the biodegradation of dodecylbenzene sulfonate sodium as well as atrazine and simazine (Farzaneh *et al*., 2010). According to a study by Isom *et al*., (2022), *S. maltophilia* prefers amino acids as a growth source for the development of biofilms. The ability of *S. maltophilia* to biodegrade xenobiotics is improved by its formation of biofilms.

Asok and Jisha, (2012) found that *Pseudomonas aeruginosa* generated biofilms and promoted the biodegradation of linear alkylbenzene sulfonate. Propanil degradation was recently shown to be accelerated by the biofilm development of *Acinetobacter sp.* (Oanh *et al*., 2020; Duc *et al.,* 2022). As in this investigation, Haque *et al*., (2022) found the biofilm-producing bacteria *Acinetobacter haemolytics* ES52G from different ecosystems. In this study, the bacterial isolate PTEB2 (*Chryseobacterium cucumeris)* lacks the potential to create biofilms. However, a study by Satti *et al.,* (2017), conducted in a different ecosystem found that *Chryseobacterium* sp. and *Pseudomonas aeruginosa* can both form biofilms and break down polylactic acid (PLA) at 30 °C. Lima *et al*., (2020) reported that *Acinetobacter sp*. and *Pseudomonas sp*. are likely to have genes that direct the development of biofilms. With the help of biofilm, *Bacillus* sp. E5 and *Pseudomonas sp*. C1 improved the biodegradation efficacy against OP fungicide (Kwak *et al*., 2013). Duc *et al*., (2022) isolated biofilm-forming *Bacillus sp. DT1* that breaks down carbofuran. By acting as a biocatalyst and destroying xenobiotic substances, biofilm eliminates them from the environment. This could be the result of the attached microbial biomass receiving more time and constant exposure to the pollutant, enabling it to adapt and break down the contaminant than suspended bacteria. All the above quoted studies and the findings of the present study emphasise the biofilm forming ability of different bacterial strains and the enhancement of the degradation process.

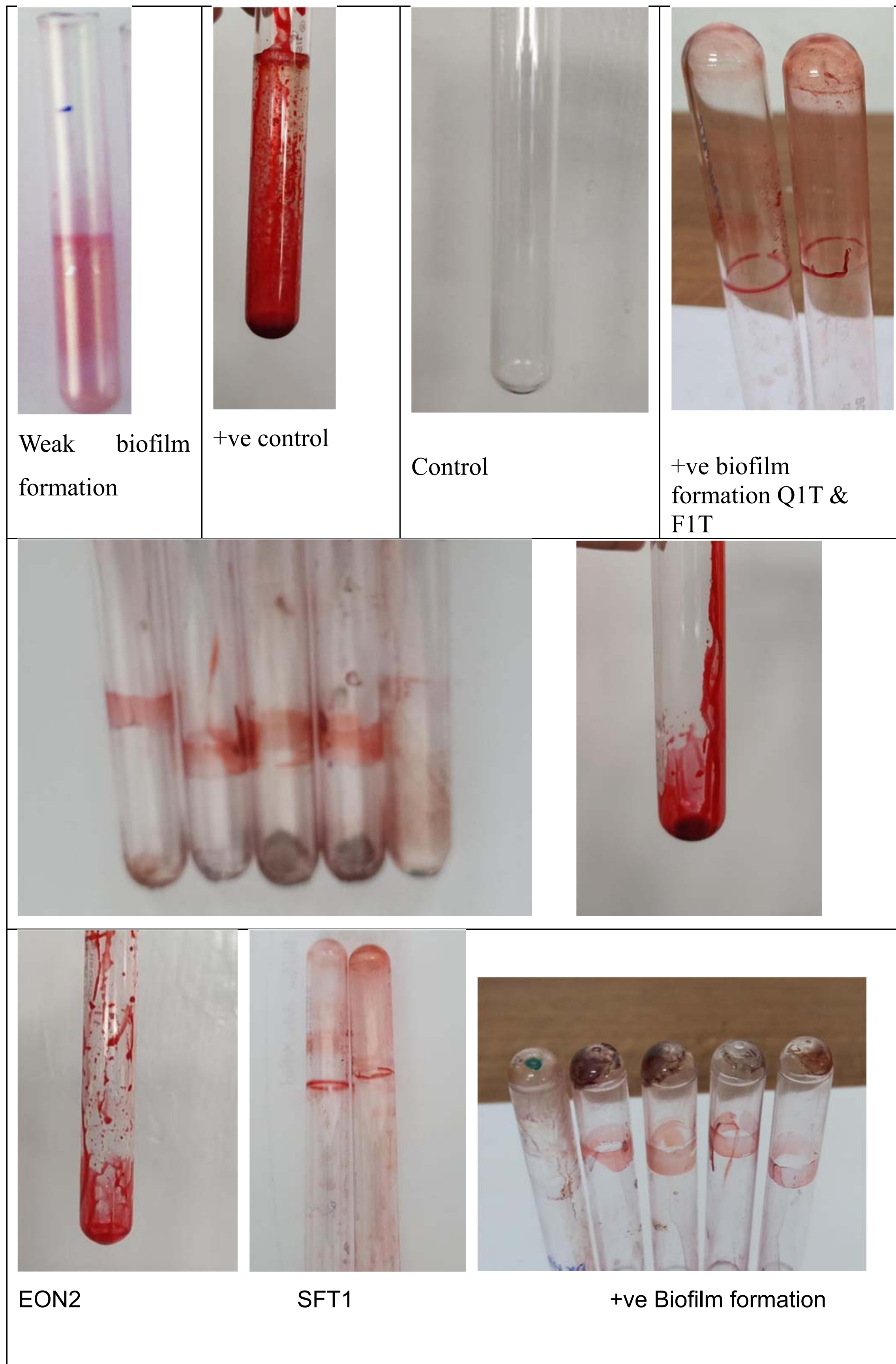


Figure 4.25 Biofilm formation in Tube Method.

4.3 Growth of bacterial isolates in different carbon and nitrogen sources

The two main processes by which microorganisms degrade pesticides are catabolism and – co-metabolism. Catabolism is the process by which bacteria use pesticides as a source of energy, specifically as a carbon, phosphate, or nitrogen source. Microorganisms can use pesticides as more than one source, such as C, N, or P, or they can use it as a single source. Pesticide breakdown supported by other metabolic processes occurring inside the microorganisms is another mechanism promoting the biodegradation of pesticides. This metabolic activity is known as co-metabolism. The method/pathway that the different bacteria adapt to mineralize a given pesticide is determined by the mechanism of metabolism of the pesticide. Nutrients act as an energy source for microorganisms, which might lead to their growth. Organic and inorganic compounds that can pass through the cytoplasmic membrane are known as nutrients. Microbes require nutrients as sources of carbon, nitrogen, certain inorganic ions, vitamins, and water. Microbes require carbon sources for growth and development. In addition to maintaining cell integrity and producing enzyme and protein catalysts, Hogg (2013) claims that carbon sources have a significant impact on transcription, the encouragement of biosynthesis, the availability of nutrients, pH, and the generation of antimicrobial chemicals. The results are depicted in the Table 4.22 to 4.35.

4.3.1 Growth of bacterial strains in different carbon sources

When cultured on a mixture of two carbon sources, bacteria exhibit one of two growth behaviours: either they consume the two sources sequentially, one after the other (diauxic), or they consume them simultaneously, called co-utilization. In the present study, the effects of several carbon sources (one source was a respective pesticide) on the growth response of all the tea garden soil bacterial isolates under shaking culture conditions were examined. Glucose is the most reliable carbon source for the growth of bacterial cells. Therefore, the growth response of bacterial isolates in the media with both respective pesticides and glucose was also observed. All the tea plantation bacterial isolates were grown in the presence of different carbon sources (Glucose, Galactose, Maltose, sucrose, and respective pesticides). The growth achieved was measured spectrophotometrically (λ600nm).

The bacterial isolates DRNB1, EON2, and TXM1 *(Stenotrophomonas maltophilia)* demonstrated maximum growth in glucose (1.99±0.002) and minimum growth in maltose (1.56±0.56). The least growth was observed in the control sample (0.25±0.01) without any kind of carbon source. The media with only the pesticides deltamethrin, thiamethoxam, and ethion exhibited bacterial growth at the range of1.89±0.21 to 1.92±0.25 in liquid MSM within 10 days of incubation. The bacterial growth in media with both the glucose and respective pesticides (deltamethrin, thiamethoxam, and ethion) the maximum growth observed was in the range of1.61±0.01 to 1.74±0.02, which is less than the bacterial growth expressed in the media with glucose and pesticide alone.. This could be attributed to the preference of bacterial strains DRNB1, TXM, and EON2 for glucose and competition between energy sources.

Table 4.22: Growth of *Stenotrophomonas maltophilia* in different carbon sources

Sl.no	**Carbon source**	**OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)**
1.	Control	0.25± 0.001
2.	Glucose	1.99± 0.21
3.	Galactose	1.85 ± 0.03
4.	Sucrose	1.92 ± 0.15
5.	Maltose	1.56± 0.56
6.	Deltamethrin	1.89± 0.21
7.	Thiamethoxam	1.92± 0.25
8.	Ethion	1.78 ±0.21
9.	Glucose+ Deltamethrin	1.61± 0.01
10.	Glucose+thiamethoxam	1.52 ± 0.02
11.	Glucose+Ethion	1.74 ± 0.02

The bacterial strain SFN1 (*Stenotrophomonas [Pseudomonas] geniculata*) expressed maximum growth in the presence of carbon source glucose (1.99 ± 0.21) and minimum growth in the presence of maltose (1.56 ± 0.21). The bacterial growth in the control sample without any carbon sources was 0.31± 0.11. The growth in the MSM with pesticide spiromesifen was 1.96 ± 0.11. The growth observed in the MSM with both glucose and spiromesifen was 1.89 ± 0.02, within 10 days of incubation period. This could be attributed to the competition or preference of *S.geniculata* for glucose

and spiromesifen for its growth. It utilizes spiromesifen as its sole carbon source in the absence of glucose.

Table 4.23: Growth of bacterial strain *Stenotrophomonas geniculate* in different carbon sources

SL.No	Carbon source	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Control	0.31± 0.11
2.	Glucose	1.99 ± 0.21
3.	Galactose	1.85 ± 0.11
4.	Sucrose	1.92 ± 0.32
5.	Maltose	1.56 ± 0.21
6.	Spiromesifen	1.96 ± 0.11
7.	Glucose+Spiromesifen	1.89 ± 0.02

The bacterial strain FXE1 (*Pseudomonas aeruginosa*) exhibited maximum growth in the presence of galactose (2.02± 0.28), and minimum growth was observed in the media with sucrose (1.65± 0.5). The bacterial growth in the MSM with pesticide fenpyroximate was 1.98± 0.31. The least bacterial growth was shown by the control sample (0.15± 0.12) without any kind of carbon sources. The bacterial growth in the MSM with both glucose and spiromesifen was 1.78± 0.01 during 10 days of incubation time.

Table 4.24: Growth of bacterial strain *Pseudomonas aeruginosa* in different carbon sources

SL.No	Carbon source	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Control	0.15± 0.12
2.	Glucose	1.91± 0.25
3.	Galactose	2.02± 0.28
4.	Sucrose	1.65± 0.5
5.	Maltose	1.21± 0.29
6.	Fenpyroximate	1.98± 0.31
7.	Glucose+Fenpyroximate	1.78± 0.01

The bacterial strain GLYB2 (*Acinetobacter baumannii*) expressed maximum growth in media with sucrose (2.86± 0.31). The minimum growth was observed in the presence of glucose (1.21 ±0.2). Maltose-induced bacterial growth was 2.52± 0.58, which is somewhat comparable to that induced by sucrose. The least growth was observed (0.31± 0.001) in the control sample. The bacterial growth in the MSM with glyphosate was 2.54 ±0.87, which is also comparable with the growth induced by sucrose. The bacterial growth in the MSM with both sucrose and glyphosate was 2.01

± 0.01 after 10 days of incubation. The bacterial strain PTEB2 (*Chryseobacterium cucumeris*) showed maximum growth in the MSM with glucose (1.96 ± 0.02), and minimum growth in the media with maltose (0.98± 0.015). The least growth was observed in the control (0.23± 0.01) sample without any carbon sources. The bacterial growth in the MSM with propargite was 1.84 ± 0.006. The bacterial growth in the MSM with both propargite and glucose was 1.81 ± 0.001 after 10 days of the incubation period. The results suggest that *Chryseobacterium* prefers glucose more than other carbon sources for its growth. *Chryseobacterium* sp. prefers galactose (1.72± 0.51) for its growth.

Table 4.25: Growth of bacterial strain *Acinetobacter baumannii* in different carbon sources

SL.No	Carbon source	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Control	0.31± 0.001
2.	Glucose	1.21 ±0.2
3.	Galactose	1.61± 0.55
4.	Sucrose	2.86± 0.31
5.	Maltose	2.52± 0.58
6.	Glyphosate	2.54 ±0.87
7.	Glucose+Glyphosate	2.01 ± 0.01

Table 4.26: Growth of bacterial strain *Chryseobacterium cucumeris* in different carbon sources

SL.No	Carbon source	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Control	0.23± 0.01
2.	Glucose	1.96 ± 0.02
3.	Galactose	1.72± 0.51
4.	Sucrose	1.54 ± 0.005
5.	Maltose	0.98± 0.015
6.	Propargite	1.84 ± 0.006
7.	Glucose+propargite	1.81 ± 0.001

The bacterial strain SFT1 *Bacillus subtillis* exhibited maximum growth in MSM with (1.81± 0.11) glucose, and minimum growth was observed in the media with maltose (0.96± 0.21). The least growth was observed in the control (0.34± 0.21) sample. The growth in media with spiromesifen was 1.85± 0.01. The bacterial growth in the MSM with both glucose and spiromesifen was 1.79± 0.001 after 10 days of incubation. The results suggest that *Bacillus subtilis* strain SFT1 prefers spiromesifen slightly more than glucose for their growth.

Table 4.27: Growth of the bacterial strain *Bacillus subtilis* in different carbon sources

SL.No	Carbon source	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Control	0.34± 0.21
2.	Glucose	1.81± 0.11
3.	Galactose	1.76 ± 0.3
4.	Sucrose	1.62 ± 0.56
5.	Maltose	0.96± 0.21
6.	Spiromesifen	1.85± 0.01
7.	Glucose+Spiromesifen	1.79± 0.001

The bacterial strains F1T and Q1T (*Paenibacillus alvei*) exhibited maximum growth in the media with glucose (2.57 ±0.015), and minimum in the media with sucrose (0.89 ±0.01). The least growth was observed in the control sample (0.35 ±0.001). The bacterial growth observed in the media with fenpyroximate was 1.82± 0.003, and with quinalphos was 2.01± 0.301, after the incubation of 10 days. In the media containing galactose (1.98 ±0.01), both strains showed moderate growth. The bacterial growth was 1.95± 0.05 in the media with glucose and quinalphos. The bacterial growth was 1.68 ±0.001 in the media with glucose and fenpyroximate. The study suggests that *Paenibacillus alvei* prefer quinalphos more than the pesticide fenpyroximate for their growth.

Table 4.28: Growth of the bacterial strain *Paenibacillus alvei* in different carbon sources.

SL.No	Carbon source	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Control	0.35 ±0.001
2.	Glucose	2.57 ±0.015
3.	Galactose	1.98 ±0.01
4.	Sucrose	0.89 ±0.01
5.	Maltose	1.76 ± 0.02
6.	Fenpyroximate	1.82± 0.003
7.	Quinalphos	2.01± 0.301
8.	Glucose+Fenpyroximate	1.68 ±0.001
9.	Glucose+Quinalphos	1.95± 0.05

According to recent studies, when a carbon source is combined with a different set of carbon compounds, the bacteria tend to co-utilize both sources and grow more quickly than they would with either source alone. Hermsen *et al.,* (2015), Peyraud *et al*., (2012), and Aidelberg *et al*., (2014), observed that when mixing two carbon sources

that are both members of the same Group, the bacteria often utilize the preferred source (with the greater growth rate) first.

Several researchers reported the growth of different bacterial strains in various carbon sources. The *Pseudomonas* produces the highest siderophore in the presence of carbon source glucose (Advinda *et al*.,2018). The bacterium isolated by Kumar *et al*., (2017), VITVK5, grew rapidly in the presence of sucrose in the media. Unlike the findings in this study, *Stenotrophomonas maltophilia* prefers amino acids over carbon sources for the development of its biofilms and carboxylate sources for the process of breakdown (Jia *et al*., 2019). Cycon *et al*.,(2009) reported that *Pseudomonas putida* expressed increased growth in the presence of glucose source. Most pesticides need an extra carbon source for their degradation. The degradation of quinalphos by *Bacillus subtillis* is improved by the presence of additional carbon source glucose, and the degradation of Chlorpyrifos by *Paracoccus sp.* is also increased by the presence of carbon sources (Xu *et al*.,2005). At the same scenario, Karpouzas and Walker (2000) it was found that the degradation of ethoprophos by *Pseudomonas sp.* is not influenced by the presence of carbon sources.. According to Palleroni and Bradbury's research findings (1993), *S. maltophilia* can use glucose as its only carbon source, which is consistent with the findings of the current investigation. *Stenotrophomonas* isolates demonstrated variation in their carbon utilization characteristics in another study by Berg *et al*., (1999). The findings of the current study show that all the isolates isolated exhibited maximum growth in the presence of different carbon sources. They also utilised pesticides as their sole energy source in the absence of carbon sources. It is also inferred that the bacterial isolates degrade their respective pesticides by metabolizing them for their growth.

4.3.2 Growth of bacterial strains in different Nitrogen sources

Maximizing the growth rate is crucial for the survival and fitness of unicellular organisms. When compared to other nitrogen sources, such as amino acids, bacteria prefer ammonia, which supports a rapid growth rate in *E. coli* (Reitzer, 2003). Nitrogen (N) is a fundamental component of all living things and is required for the synthesis of amino and nucleic acids.

In the present study, the effects of several nitrogen sources (one source was a respective pesticide) on the growth response of all the tea garden soil bacterial isolates

under shaking culture conditions were examined. The tea plantation bacterial isolates were grown in the presence of different nitrogen sources (Ammonium nitrate, ammonium chloride, urea, Ammonium sulphate and respective pesticides). The growth achieved was measured spectrophotometrically (λ600nm).

The bacterial strains DRNB1, EON2, and TXM1 exhibited high growth in the media with ammonium nitrate (1.96± 0.12), and minimum growth in the media with urea (1.23 ±0.45). . The bacterial growth in the MSM with ammonium sulfate was 1.76 ± 0.05, after the incubation of 10 days.

Table 4.29: Growth of bacterial strain *Stenotrophomonas maltophilia* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	1.96± 0.12
2.	Ammonium chloride	1.65 ± 0.02
3.	Ammonium sulphate	1.76 ± 0.05
4.	Urea	1.23 ±0.45

The bacterial strain SFN1 (*Stenotrophomonas [Pseudomonas] geniculata*) expressed maximum growth in the media with ammonium nitrate (1.96 ±0.21), and minimum growth was in the media with (1.23± 0.15) Urea. The least growth was observed in the control sample, without any kind of energy sources. The bacterial strain FXE1 (*Pseudomonas aeruginosa*) expressed maximum growth in the MSM with (1.98± 0.11) ammonium sulphate, and the minimum was observed in the media with urea (0.89 ±0.12); the bacterial growth of about 1.65± 0.11 was observed in the ammonium nitrate media.

The bacterial strain GLYB2 (*Acinetobacter baumannii*) preferred the nitrogen source ammonium nitrate (2.09± 0.23). The minimum growth was observed in the media with urea (0.68± 0.21). The least growth was observed in the control sample. The bacterial strain also prefers ammonium sulphate and exhibited a growth of 1.81± 0.11, after the incubation of 10 days. The bacterial strain PTEB2 (*Chryseobacterium cucumeris*) exhibited maximum growth in ammonium nitrate (2.05 ±0.01) followed by ammonium chloride (1.86± 0.015). the minimum growth was observed in the media with ammonium sulfate (0.76± 0.005). the bacterial SFT1 (*Bacillus subtilis*) expressed maximum growth in ammonium sulphate (1.84 ±0.14), and minimum growth in media with ammonium chloride (1.05± 0.11); the least growth was

observed in the control sample. It also exhibited a growth of 1.62 ± 0.22 in the media with urea.

The bacterial strains F1T and Q1T (*Paenibacillus alvei*) showed maximum growth in the media with ammonium chloride (2.05 ± 0.15), and minimum growth in the media with urea (0.78 ± 0.01); they exhibited a growth of 1.86 ± 0.006 in the media with ammonium sulphate. The study results reveal that *Stenotrophomonas maltophilia*, *Stenotrophomonas geniculate*, *Acinetobacter baumannii*, *Chryseobacterium cucumeris* prefer ammonium nitrate as their nitrogen source for maximum growth, *Pseudomonas aeruginosa* prefers ammonium sulphate, and *Paenibacillus alvei* prefers ammonium chloride for their growth.

There have been several recent studies on how different nitrogen sources affect bacterial development. The additional nitrogen sources in the media encourage the biomass production of microorganisms. Alternative nitrogen sources, including amino acids, are thought to be poor nitrogen sources since they sustain substantially slower growth rates (Ikeda *et al*., 1996). According to Costa *et al*. (2002), organic nitrogen sources are better able to support the rapid growth of bacteria and high cell yields than inorganic nitrogen sources. According to Chai and Adnan, (2018), *Pseudomonas aeruginosa* produced more cellulose when yeast and beef extracts were present. Coban and Biyik, (2011) and Kazim (2015) provided evidence that corroborate the finding of this study that yeast extracts promote and accelerate bacterial growth. According to Singh (1971), *Bacillus sp*. may thrive in both ammonium glutamate and ammonium nitrate. In the presence of nitrogen sources, the growth of many dairy starter cultures and lactic acid bacteria is accelerated (Proust *et al*., 2019). The ability of the bacterial strains to degrade substances also increases in the presence of nitrogen sources (Chu and Cohen, 1998).

Table 4.30: Growth of the bacterial strain *Stenotrophomonas geniculata* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	1.96 ±0.21
2.	Ammonium chloride	1.23± 0.15
3.	Ammonium sulphate	1.76 ± 0.12
4.	Urea	1.23 ± 0.01

Table 4.31: Growth of the bacterial strain *Pseudomonas aeruginosa* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	1.65± 0.11
2.	Ammonium chloride	1.21 ± 0.13
3.	Ammonium sulphate	1.98± 0.11
4.	Urea	0.89 ±0.12

Table 4.32: Growth of the bacterial strain *Acinetobacter baumannii* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	2.09± 0.23
2.	Ammonium chloride	1.65 ± 0.01
3.	Ammonium sulphate	1.81± 0.11
4.	Urea	0.68± 0.21

Table 4.33: Growth of the bacterial strain *Chryseobacterium* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	2.05 0.01
2.	Ammonium chloride	1.86 0.015
3.	Ammonium sulphate	0.76 0.005
4.	Urea	1.69 ± 0.01

Table 4.34: Growth of the bacterial strain *Bacillus subtilis* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	1.52 ± 0.05
2.	Ammonium chloride	1.05 0.11
3.	Ammonium sulphate	1.84 0.14
4.	Urea	1.62 0.22

Table 4.35: Growth of bacterial strain *Paenibacillus alvei* in different nitrogen sources

Sl.No	Nitrogen sources	OD @ 600nm (After 10 days in aqueous MSM) (Mean±SD)
1.	Ammonium nitrate	1.65 ± 0.12
2.	Ammonium chloride	2.05 ±0.15
3.	Ammonium sulphate	1.86 ± 0.006
4.	Urea	0.78± 0.01

To maintain the physiological and metabolic activity of bacteria, nitrogen supplies are crucial. According to a study by Huang *et al*.,(2023), the inclusion of the inorganic nitrogen source ammonium sulphate increases the capacity of *S.maltophilia* to produce biomass. The fact that urea is present in the media indicates which is the least efficient. Additionally, *Stenotrophomonas maltophilia* was able to degrade - cembratiene-4,6,-diol more rapidly due to ammonium sulphate. Ammonium, a nitrogen source, facilitates the development and transformation of *Stenotrophomonas maltophilia* W-6 (Wang *et al*., 2016). He *et al*.,(2023) reported that ammonium is the most preferred nitrogen source of *Bacillus sp.* They also found that *Bacillus* sp. also utilizes urea, nitrite, and nitrate for its growth. According to Britta Jurgen *et al.* (2008), although glutamine and arginine are thought to be *Bacillus subtilis* preferred nitrogen sources, the combination of ammonia and casamino acids also helps the bacteria produce more biomass. According to Wang *et al*. (2016), bacteria primarily prefer ammonia for growth. According to Li *et al*. (2023), *Bacillus subtilis* favours ammonium chloride for growth. According to Yang (2014) and Lee *et al*. (2004), *Acinetobacter sp*. favours ammonium, nitrate, and urea as the only nitrogen sources. *Chryseobacterium sp*. showed the greatest growth in the media with ammonium chloride, according to Pradyut *et al*., (2014) in a study of bacterial growth in several nitrogen sources including sodium nitrite, sodium nitrate, and ammonium chloride.

4.4 Degradation studies

The pesticide degradation studies were done in both liquid MSM and soil samples under laboratory conditions. The concentration of the pesticides was obtained through the GC-MS quantitative analysis. The metabolites in MSM samples were determined through the GC-MS qualitative analysis. For pesticide concentration determination a sample of either 10 ml of MSM or 10 gm of soil was taken. MSM samples were diluted with 20 ml of deionized water and extracted twice with using 10 ml of appropriate solvents each time. The extractions were carried out on a rotary shaker for 30 mins. Soil samples were extracted with 20ml of suitable solvents in a rotary shaker for 1 hour. Afterward, the extracts were subjected to dehydration using anhydrous sodium sulphate (Na_2SO_4) and then evaporated to dryness under a continuous stream of N_2 gas at 45^oC using a rotary evaporator. Following that, the solution was diluted to total amount of 10 ml using either hexane or acetone and set aside for

chromatographic analysis. The degradation percentage was determined by using the Equation 5 illustrated in chapter 3 section 3.9.5 .

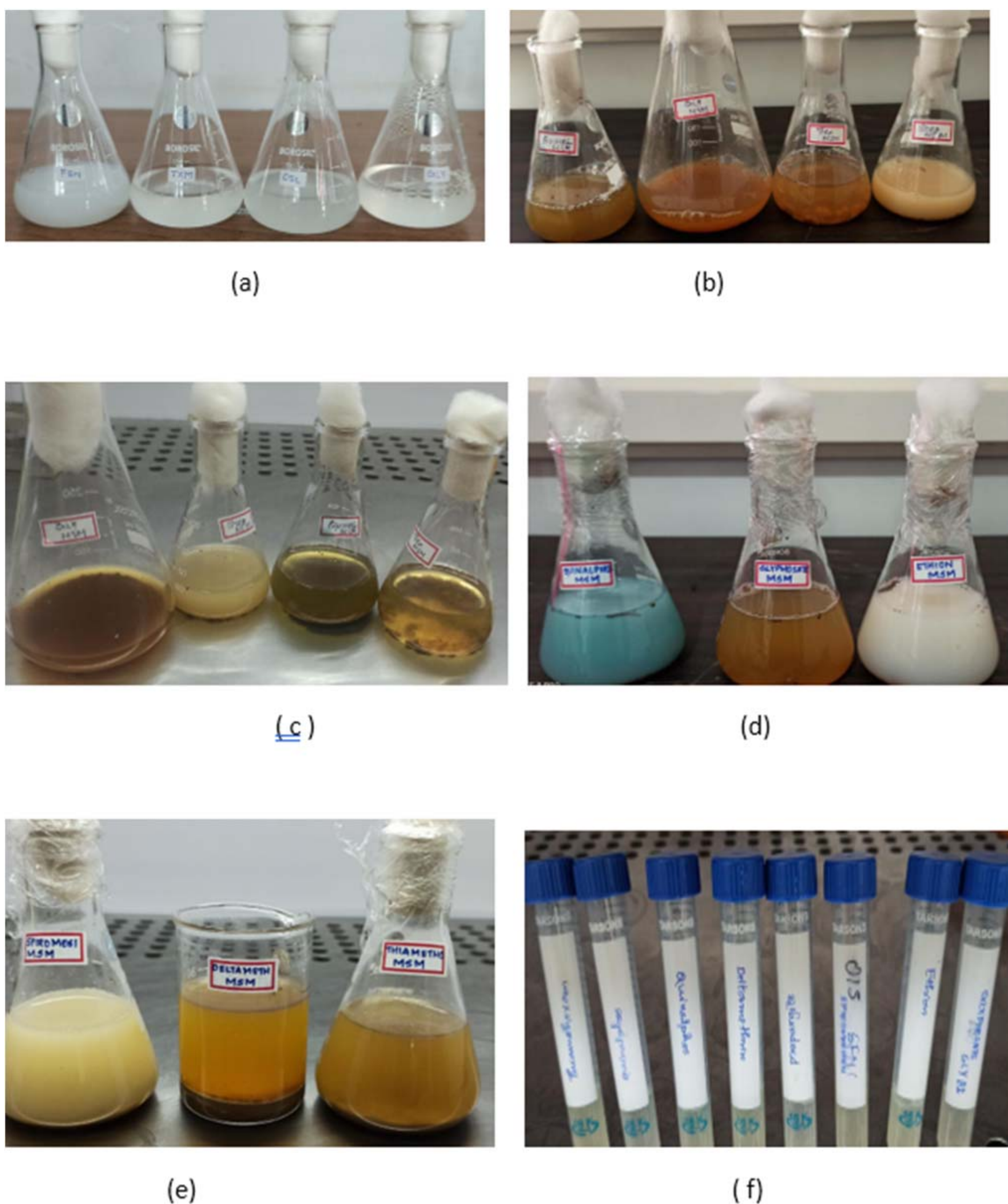


Figure 4.26 The experimental setup of degradation studies of different pesticides in liquid MSM. (a,b,c,d,& e) liquid MSM with pesticides or enrichment culture & (f) preparation of samples for the centrifugation process. (a), (b), (c), (d) denotes the liquid MSM with deltamethrin, Thiamethoxam, glyphosate, fenpyroximate, and spiromesifen.

4.4.1 Degradation studies in liquid MSM and optimization of parameters

For the optimization of parameters, pH at five different levels (pH 5 to pH 9), the temperature at five different levels (15°C to 40°C), and bacterial inoculum size from

0.5 to 2.5(OD @600nm) were selected. Degradation studies were done at every level of parameters, and by applying a single factor test or one-way ANOVA three best levels of each parameter were selected and applied the Taguchi optimization method. The signal-to-noise ratio (S/N) larger is better was selected for the optimization of the parameters.

4.4.1.1 Degradation of deltamethrin by *S. maltophilia* strain DRNB1 in MSM

The degradation of deltamethrin by *S. maltophilia* strain DRNB1 was analysed at different temperatures (15°C to 40°C), at different pH (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain DRNB1 was also analysed at different temperatures and pH. The deltamethrin was degraded at all the selected temperatures at different rates. The maximum degradation mean value of 96.03 ± 1.85% was observed at 30°C. A Degradation mean value of 94.46 ± 1.9 was observed at 40°C. The low degradation of deltamethrin was observed at temperatures 15° and 20°C with mean values of 10.65 ±1.2% and 22.18 ± 0.15% respectively. The analysis was based on different pH, the maximum degradation was observed at pH 7 with a mean value of 96.56 ± 1.9%. The degradation with a mean value of 95.5± 1.9% and 92 ± 1.8% was observed at pH 6 and pH 8 respectively. It can be inferred from this that DRNB1 prefers neutral pH for the metabolization of deltamethrin. When different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 2 (OD @ 600nm) with a mean value of 97.06 ± 1.9%. This may be because the rate of deltamethrin degradation increases with an increase in bacterial growth. In this study it was noted that DRNB1 adapts to a wide range of temperature and exhibits growth at the selected temperatures (15° to 40°C). Its growth is only low at 15°C and 20°C with a mean value of 0.12 and 0.15 respectively. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 1.98 ± 0.15. DRNB1 can grow in the pH range of 5 to 9. The highest OD value of 1.95 was observed at the pH 7 and the lowest at the pH 5 with a value of 0.3. The degradation rate of deltamethrin in the control flasks (MSM and deltamethrin, without the strain DRNB1) was about 15% after 10 days of incubation. Wenyuan et al., (2018) isolated deltamethrin degrading *S.maltophilia* strain XQ08 from a different ecosystem which was the agricultural soils of Guizhou Province. They found that the maximum degradation of deltamethrin by strain XQ08

is observed at the temperature 35°C and pH 8. However, in the present study, the maximum degradation was observed at the temperature of 30°C and pH 7. This could be attributed to the change in ecosystem and climatic conditions. study. Nevertheless, the study by Wenyuan et al., (2018) provides valuable insights and also makes it clear that *S.maltophilia* can degrade deltamethrin as found in the present study.

Response Table for Means

Level	Temperature	pH	inoculum size
1	96.03	93.29	95.15
2	92.46	95.89	93.75
3	94.25	93.55	93.83
Delta	3.57	2.61	1.40
Rank	1	2	3

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	inoculum size
1	39.65	39.39	39.56
2	39.32	39.63	39.44
3	39.48	39.42	39.44
Delta	0.33	0.24	0.13
Rank	1	2	3

Figure 4.27 : Design summary of Taguchi Array with factors and no of Runs(a), Response table showing the delta ranking of different parameters

To optimise deltamethrin breakdown by DRNB1, single-factor, and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L $9[3]^3$) was designed with the following factors: temperature (A-30, 35, 40°C), solution pH (B – 6.0, 7.0, 8.0), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm). The strain DRNB1 was inoculated into the flasks containing MSM and 100ppm of deltamethrin. The experiment was carried out in triplicate and MSM along with deltamethrin flasks without strain DRNB1 was treated as a control sample. The Taguchi OA test results of the optimum parameters for degrading deltamethrin are shown in Table 4.43. The optimum parameters for degrading deltamethrin were selected based on Signal to Noise ratio (fig 4.27) by selecting the option ‘larger is better’ value. Based on the above test results the parameter temperature influences the deltamethrin removal rate the most, followed by pH, and the inoculum size (A>B>C). Based on the main effect plots, the optimum temperature was 30°C, pH was 7, and inoculum size was 1.5 (OD @600nm) ($A_1B_2C_1$). Due to the interactions of significant factors and their levels in

varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 h) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that temperature has maximum effect on the biodegradation of deltamethrin while the inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 98.58% (S/N – 39.88) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 30°C (Level 1), pH 7 (Level 2), and inoculum density 1.5 (Level 1). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed deltamethrin degradation was 98% which is significantly similar to the predicted value (98.58%) at 95% level of confidence.

Recent studies have focused on the degradation ability of *Stenotrophomonas* sp. in different ecosystems. Chen et al., (2011) reported that *Stenotrophomonas* strain ZS-S-01 increased the dissipation rate of pyrethroid pesticide fenvalerate in soil. They also reported that the half-life of fenvalerate decreased to eight times in the presence of *Stenotrophomonas sp*. Both deltamethrin and cypermethrin are pyrethroid insecticides with particularly similar structures. Gur *et al*., (2014) reported that *S. maltophilia* strain OG2 could degrade 69.9% of 100 mg/L cypermethrin after 10 days of incubation, while *S. maltophilia* strain DRNB1 in this study degraded 97.90% 100ppm of deltamethrin after 8 days of incubation. Wu et al. (2021) also isolated S. *maltophilia XQ08* in soil samples from different ecosystems, which could degrade 63.26% of 100 mg/L deltamethrin after 5 days of incubation. They also found that the optimum parameters for the maximum degradation of deltamethrin are temperature 35°C, pH 7, and cell concentration of 5.5 x 10^{8} cfu/ml. These results suggest that *S.maltophilia* strains have the potential for the rapid degradation of pyrethroid insecticides, particularly deltamethrin. All these study reports are in agreement with the present study.

Bacteria capable of degrading deltamethrin were mostly isolated from agricultural areas where intensive pesticides were used. Akbar et al., (2015) isolated five different bacteria species *Acinetobacter calcoaceticus* MCm5, *Brevibacillus parabrevis* FCm9, *Sphingomonas* sp. Rcm6, *Bacillus megaterium* JCm2, O*chrobactrum* anthropic JCm1, and *Rhodococcus sp*. JCm5 with the potential to degrade deltamethrin. Song et al.,

(2014) studied the biodegradation ability of the *Pseudomonas aeruginosa* JO-41 strain isolated from pyrethroid contaminated soil. The main effects plot for means of deltamethrin degradation is represented in the Figure below:

Cycon *et al*. (2014) isolated deltamethrin degrading *Serratia marcescens* Del-1, Del-2 from insecticide treated soil. The other deltamethrin degrading species are *Bacillus cereus* Y1 (Zhang *et a*l., 2016), *Lysinibacillus fusiforms* ZJ6 (Hao *et al.,* 2018), *Acinetobacter baumannii* ZH-14 (Zhan *et al*., 2018). Kumral *et al.* (2020) isolated deltamethrin degrading *Lactobacillus plantarum*, which is used as a food fermenter. Such investigations provide ample validation for the findings of this study that biodegradation is the best method for the degradation of deltamethrin.

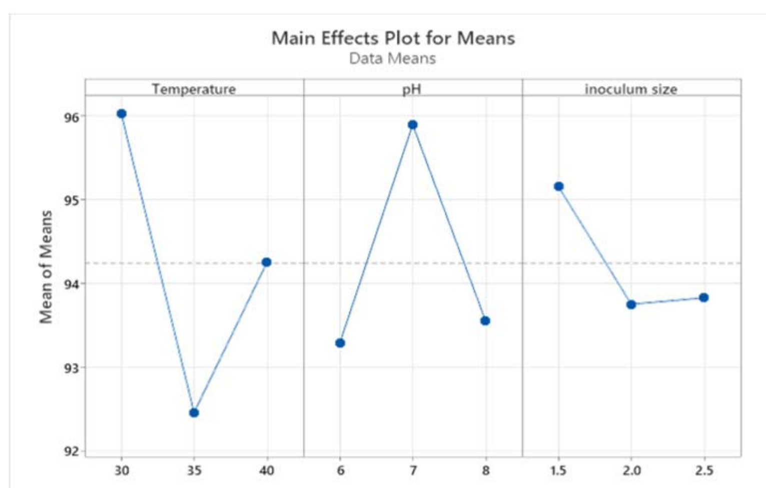


Figure 4.28: Optimization graph of parameters temperature, pH and Inoculum siz

4.4.1.2 Degradation of Thiamethoxam by *S.maltophilia* strain TXM2

Bacterial isolate TXM-2 was able to utilize thiamethoxam as the sole carbon source and showed effective growth within 2 to 6 days of incubation without any initial lag phase.TXM-2 demonstrated maximum degradation capacity by utilizing thiamethoxam in 8 days and degrading it to more than 85% within 10 days in liquid MSM. The degradation rate of thiamethoxam increased gradually as the strain entered the logarithmic phase (4-5) days, followed by the stationary phase (7 – 8 days). A decline in degradation rate was observed when the strain attained the death phase (after 10 days). Taguchi OA approach was used to determine the effect of three important variables (temperature, pH, and inoculum size) on the biodegradation of thiamethoxam by *Stenotrophomonas maltophilia* strain TXM1. The experimental

design and the response of dependent variables obtained by performing the biodegradation experiments under L-9 OA for thiamethoxam are presented in Table no:4.37. The data from Table 4.37 were processed by the Taguchi OA method of Minitab software and results were obtained from the signal-to-noise ratio "larger is better". *S.maltophilia* is also reported to degrade several xenobiotic compounds (Wu *et al*.,2021). This study provides evidence of an efficient degradation pathway of thiamethoxam by *Stenotrophomonas malto*philia strain TXM-2. Biodegradation of thiamethoxam increased with increased bacterial growth in the minimal medium. As evident from the figure 4.30, temperature was found to play the most prominent role in microbial growth followed by pH and inoculum size.

TXM-2 could utilize thiamethoxam as a sole carbon source and degrade it over a wide range of temperatures (25-30°C), pH (6-8), and inoculum size (1-2 OD @ 600nm). The purpose of using Taguchi in the present study was to optimize the best culture conditions for the maximum degradation of the thiamethoxam. The thiamethoxam degradation rate by *S.maltophilia* strain TXM-2 increased to 62.8% at pH 6 and reached the maximum at pH 7, but reduced to 23% at pH value 9 after 10 days of incubation. The degradation efficiency increased from 58% over 6 days to 86.8% over 8 days at pH 7, which suggested that thiamethoxam was easily hydrolysed in neutral to alkali solutions (pH 7 – 8). The incubation temperature greatly influenced the degradation of thiamethoxam by strain TXM-2.

The maximum degradation rate of 84% was observed at 35°C within 8 days, but this rate decreased markedly as the temperature increased above or dropped below 30°C and the degradation rate was only 32% at 40°C. The results displayed that 30°C was the optimal temperature for the degradation of thiamethoxam. To arrive at the optimal number of bacterial cells for the effective degradation of thiamethoxam the influence of different inoculum sizes ranging from 0.5 to 2.5 (AU @ 600nm) was studied. While the addition of 0.5 caused no enhancement in the degradation of thiamethoxam. The addition of 1 and 1.5 OD cells increased the substantial increase in the thiamethoxam degradation. The maximum degradation was attained at the cell concentration of 2. The changes in pH value have a significant impact on cell growth and thiamethoxam degradation. When the pH value ranges from 6 to 8, the biomass was increased gradually. While if the pH value changes from 8 to 9, the biomass decreases gradually. That is because of the highly acidic and highly alkaline

environment which is not suitable for the growth of the bacteria. A suitable pH value can increase the reaction activity and accelerate the utilization of nutrients from the medium. To achieve the maximum degradation rate, the optimum pH gradient ranges from 6 to 7. The maximum degradation rate was obtained in pH 7 media, and the degradation rate reached 84.76%. The degradation rate and bacterial growth are low at pH 5 with 37.7%.

For the maximum degradation rate, the production temperature varies from 15˚ to 40˚C.The degradation also increased too as the increase of biomass. However, when the temperature exceeded 40˚C, the degradation rate had been significantly reduced. The degradation rate is maximum, 86.03% at the temperature of 35°C. As a result, it is well proved that the optimal temperature was 35˚C. The acidity or alkalinity of medium expressed as pH affects how accessible their nutrients are to organisms. pH and temperature both have a significant effect on bacterial growth and their metabolic characteristics affecting the growth and community structure of soil bacteria strongly. Most of the bacteria have a relatively narrow pH tolerance varying between 2-3 pH units between minimum and maximum, and hence, are directly influenced by environmental pH (Singh and Iyer, 2007).

The maximum degradation with a value of 82.530% was obtained in experiment number 8 at the temperature 35°C, pH 7, and inoculum size 1 (OD@600nm). The half-life at this experiment was 4.04 days. In accordance with the Taguchi test the optimum temperature, pH, and inoculum size for thiamethoxam degradation by *S.maltophila* were 35°C, 7, and 2 respectively. According to the delta rank, temperature influences the degradation of thiamethoxam followed by pH and inoculum size. One-way ANOVA analysis ($p \leq 0.01$) revealed that pH variations have a significant effect on the growth of bacterial isolates. Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 b) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that has maximum effect on the biodegradation of thiamethoxam was temperature while the inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 83.432% (S/N – 38.5093) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 35°C (Level 3), pH 7 (Level 2), and inoculum density 2

(Level 3). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed thiamethoxam degradation was 84.59 % which is significantly similar to the predicted value (83.432%) at 95% level of confidence.

Singh and Kumar, (2003) reported that microorganisms depend majorly for their growth on environmental factors particularly on temperature. In this study, the thiamethoxam was removed from the MSM by *S.maltophilia* strain TXM2 with a degradation rate of 82.53% within 8 days of incubation. Several studies have been done on the degradation of thiamethoxam in recent years. Zhou et al., (2013) isolated thiamethoxam degrading *Ensifer Adhaerens* strain TMX-23 and studied its PGPR properties. Zhou et al., (2014) reported that soil enrichment culture rapidly degraded 96 percent of 200 mg/L thiamethoxam in mineral salt medium broth within 30 days. They isolated 31 bacterial strains and 6 strains with PGPR properties. Whereas in the present study 100mg/L of thiamethoxam was degraded in 10 days of incubation. It may be due to the difference in substrate concentration, where the increase in pesticide concentration affects the degradation time of the pesticides. Hegde et al., (2017) isolated three thiamethoxam degrading bacterial species: *Acinetobacter sp. Enterobacter sp. and Bacillus sp*. from different ecosystems. They degraded the thiamethoxam with a value of 94.72%, 90.78%, and 82.06% respectively after 15 days of incubation in MSM. Zamule et al., (2021) studied the thiamethoxam degradation ability of 6 bacterial species. They reported that *P. flurescens, P.putida, P.aeruginosa*, and *Alcaligenes faecalis* degraded thiamethoxam with a value of 67%, 65%, 52%, and 39% respectively after 15 days of incubation in MSM. They also reported that *E.coli* and *Streptococcus lactis* degraded thiamethoxam with a value of 60% and 12% respectively after 14 days of incubation. They observed that the maximum degradation occurs at a temperature of 30°C. All these studies suggest that thiamethoxam is efficiently degraded by different bacterial species within a short period, thus corroborating the findings of the present study.

Response Table for Means

Level	Temperature	pH	Inoculum size
1	62.89	68.77	71.70
2	74.14	75.78	71.06
3	78.32	70.81	72.61
Delta	15.43	7.02	1.55
Rank	1	2	3

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	Inoculum size
1	35.94	36.66	37.00
2	37.40	37.57	37.03
3	37.87	36.97	37.18
Delta	1.93	0.91	0.18
Rank	1	2	3

Figure 4.29: Response table for means and signal to noise ratios showing the delta ranking of parameters of TXM degardation

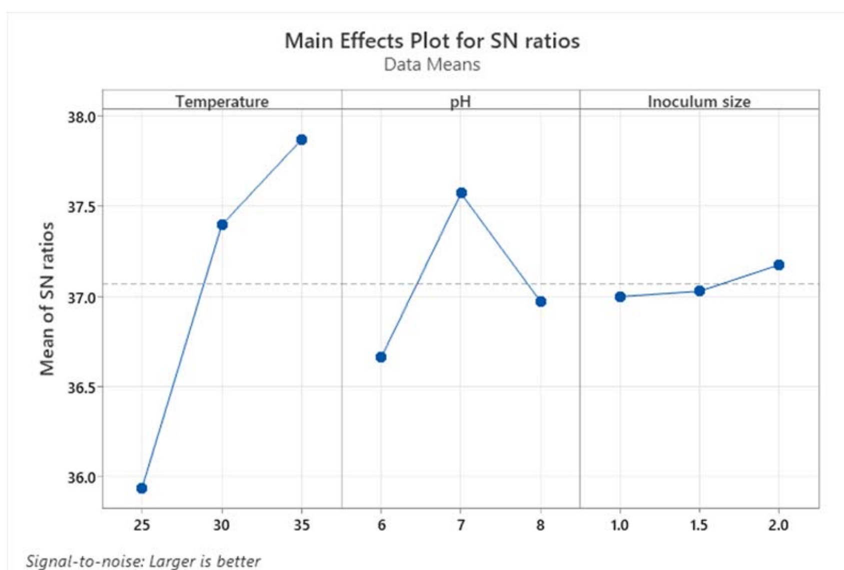


Figure 4.30: Mean effect plot of different parameters with high SN ratios of TXM degradation

In this study the kinetic modelling was studied and the thiamethoxam degradation follows first -order kinetics (Table 4.37). This study was in agreement with the report of Rana et al., (2015), and Zamule et al., (2021) who studied the kinetic modelling of thiamethoxam and found that degradation of thiamethoxam follows first - order kinetics. In comparison with previous studies of thiamethoxam degradation by bacteria, the highest value of degradation was attained by *S.maltophilia* strain TXM2 with a value of 82.53% after 8 days of incubation. So it is inferred that *S.maltophilia* strain TXM2 is extremely useful for the biodegradation of thiamethoxam.

The *Stenotrophomonas* species has been shown to degrade a variety of hazardous compounds like polycyclic aromatic hydrocarbons (Juhasz *et al*., 2000), endosulfan (Barragain-Huerta *et al*., 2007; Kumar *et al*., 2017), 4-substituted phenols (Liu *et al*., 2009), herbicide butachlor (Dwivedi *et al*., 2010), a wide range of pyrethroids such as

fenvalerate, deltamethrin, β-cypermethrin, and cyhalothrin (Chen *et a*l., 2011), acetamiprid (Tang *et al*., 2012), acrylamide (Lakshmikandan *et al*., 2014), α-endosulfan (Ozdal *et al*., 2016), and diazinon (Pourbabaee *et al.,* 2018) and diuron (Egea *et al*., 2017; Silambarasan *et al*., 2020). These studies indicate that *Stenotrophomonas sp*. has a high potential to degrade harmful substances, which validates the findings of the present study.

4.4.1.3 Degradation of Ethion by *Stenotrophomonas maltophilia* strain EON2

The degradation of ethion by *S. maltophilia* strain EON2 was analysed at different temperatures (15°C to 40°C), at different pH (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain EON2 was also analysed at different temperatures and pH. The ethion was degraded at all the selected temperatures at different rates. The maximum degradation mean value of 96.02 ± 0.025% was observed at 40°C. Degradation mean value of 94.37 ± 0.233% and 92.03±0.03% was observed at 35 and 30°C respectively. The low degradation of ethion was observed at temperatures 15 and 20°C with mean values of 21.86 ±1.6% and 24.98 ± 0.11% respectively. The analysis was based on different pH, the maximum degradation was observed at pH 7 with a mean value of 93.27 ± 0.39%. The degradation with a mean value of 91.34± 0.31% and 93.02 ± 0.02% was observed at pH 6 and pH 8 respectively. This could be attributed to the preference of strain EON2 for neutral pH for the metabolization of ethion. When different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 2 (OD @ 600nm) with a mean value of 94.57 ± 0.13%. This could be due to the reason that the rate of ethion degradation increases with an increase in bacterial growth. A degradation of ethion with a mean value of 92.09± 0.09% and 94.7 ±0.13% was attained by the addition of inoculum size 1 and 1.5 (OD@600nm). It was noted that strain EON2 adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C). Its growth is only low at 15°C and 20°C with a mean value of 0.86± 0.01 and 1.46± 0.05 respectively. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 2.55 ± 0.55. The strain EON2 can grow in the pH range of 5 to 9. The highest OD value of 2.4 ±0.04 was observed at pH 7 and the lowest at pH 5 with a value of 0.55 ± 0.015. The

degradation rate of ethion in the control flasks (MSM and ethion, without the strain EON2) was about 21% after 10 days of incubation.

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	Inoculum size
1	39.35	39.43	39.36
2	39.43	39.44	39.44
3	39.46	39.37	39.44
Delta	0.11	0.07	0.09
Rank	1	3	2

Response Table for Means

Level	Temperature	pH	Inoculum size
1	92.78	93.65	92.89
2	93.69	93.77	93.76
3	94.00	93.05	93.81
Delta	1.21	0.72	0.92
Rank	1	3	2

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Figure 4.31: Response tables for SN ratios and means of Ethion degradation by EON2

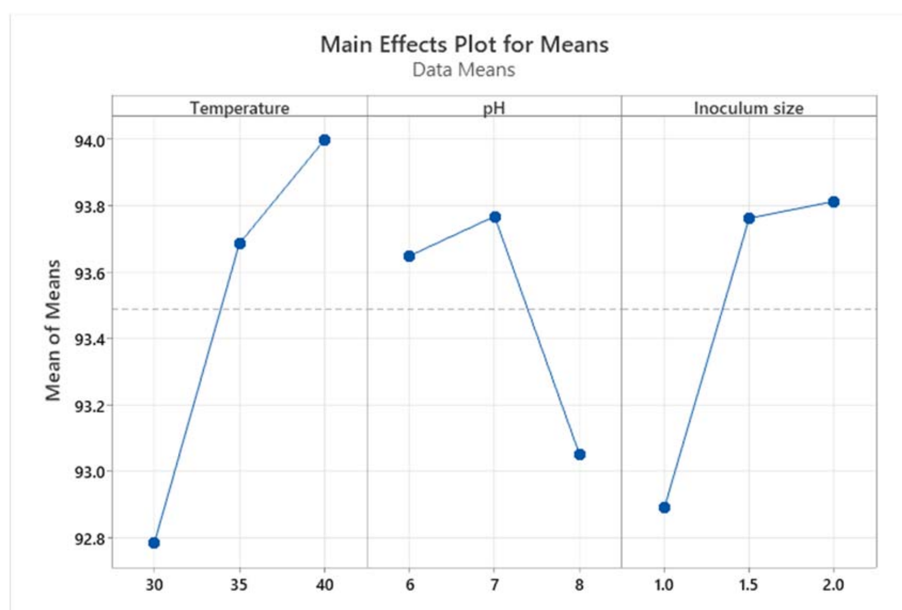


Figure 4.32: Main effect plots showing the parameter optimization of ethion degradation by EON2

To optimise ethion degradation by strain EON2, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single-factor test findings, an orthogonal test (L $9[3]^3$) was designed with the following factors: temperature (A-30, 35, 40°C), solution pH (B – 6.0, 7.0, 8.0), and inoculum size (C – 1, 1.5, 2.0 OD@600nm) (figure 4.31). The strain EON2 was inoculated into the flasks containing MSM and 100ppm of ethion. The experiment was carried out in triplicate and MSM along with ethion flasks without

strain EON2 was treated as a control sample. The experiment no 7 with factors temperature 40°C, pH 6, and inoculum size 2 (OD @ 600nm) resulted in the highest degradation with a value of 94.486% as well as S/N ratio (39.5073) while minimum degradation (92.380%) and S/N ratio (39.3116) were observed in experiment No 1 (Table 4.42). The optimum parameters for degrading ethion were selected based on Signal to Noise ratio by selecting the option 'larger is better' value. It can be concluded based on the above test results that the parameter temperature influences the ethion removal rate the most, followed by inoculum size, and pH (A>C>B). On the basis of the main effect plots, the optimum parameters suggested by Taguchi were temperature 40°C, pH was 7 and inoculum size was 2 (OD @600nm) ($A_3B_2C_3$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 g) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that has maximum effect on the biodegradation of ethion was temperature while pH was the least contributing factor. As predicted by the Taguchi DOE, 94.60% (S/N – 39.518) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 40°C (Level 3), pH 7 (Level 2), and inoculum density 2 (Level 3). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed ethion degradation was 94.72 % which is significantly similar to the predicted value (94.60%) at 95% level of confidence.

Studies on ethion degradation by microorganisms are very limited. Bishnu et al., (2012) studied the ethion degradation in tea soils and reported that tea soils are dependent on temperature, soil type and application rate of the pesticide. Foster et al., (2004) isolated two bacterial species, *Pseudomonas* and *Azospirillum*, with the ability to degrade ethion from Australian cattle dip locations. These isolates metabolised ethion at the rate of 58% and 70% respectively. They also reported that ethion degrades more quickly because it lacks an aromatic or heterocyclic group. According to the results of the research to date, this is the only study report available on the degradation of ethion by soil bacteria. In the present study, the half-life of ethion was 2.41 days at 40°C. Both Dierberger and Pfeuffer, (1983), and Foster et al., (2004) observed that the half-life of ethion was 20.8 weeks in buffered distilled water at

30°C. This difference in findings of the present study may be due to the difference in bacterial strain, temperature, and ecosystem. From the present study, it is inferred that *S.maltophilia* strain EON2 is highly capable of the degradation of ethion at any different climatic conditions.

4.4.1.4 Degradation of Spiromesifen by *Stenotrophomonas [Pseudomonas] geniculata* strain SFN1

The degradation of Spiromesifen by *Stenotrophomonas [Pseudomonas] geniculata* strain SFN1 was analysed at different temperatures (15°C to 40°C), at different pH (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain SFN1 was also analysed at different temperatures and pH. The spiromesifen was degraded at all the selected temperatures at different rates. The maximum degradation mean value of 90.03 ± 0.05% was observed at 35°C. Degradation mean value of 80.07 ± 0.06% and 77.61±0.04% was observed at 40°C and 30°C respectively. The low degradation of spiromesifen was observed at temperatures 15°C and 20°C with mean values of 16.85 ±0.02% and 21.26 ± 0.1% respectively. In the analysis based on different pH, the maximum degradation was observed at pH 7 with a mean value of 89.1 ± 0.1%. The degradation with a mean value of 79.25± 0.03% and 76.13 ± 0.12% was observed at pH 8 and pH 6 respectively. This could be attributed to the fact that strain SFN1 prefers neutral pH for the metabolization of spiromesifen. When different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 2 (OD @ 600nm) with a mean value of 81.3 ± 0.15%. This could be because the rate of spiromesifen degradation increases with an increase in bacterial growth. A degradation of spiromesifen with a mean value of 76.18± 0.07% and 70.33 ±0.3% was attained by the addition of inoculum sizes 1.5 and 2.5 (OD@600nm). It was noted that strain SFN1 adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C). Its growth is only low at 15°C and 20°C with a mean value of 0.65± 0.03 and 0.90± 0.01 respectively. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 2.0 ± 0.01. The strain SFN1 can grow in the pH range of 5 to 9. The highest OD value of 2.02±0.02 was observed at the pH 7 and the lowest at the pH 5 with a value of 1.47 ± 0.01. The degradation rate of spiromesifen in the control flasks (MSM and

spiromesifen, without the strain SFN1) was about 28.5 ± 0.23% after 10 days of incubation.

To optimise spiromesifen degradation by strain SFN1, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L $9[3]^3$) was designed with the following factors: temperature (A-30, 35, 40°C), solution pH (B – 6.0, 7.0, 8.0), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm)(Figure 4.33). The strain SFN1 was inoculated into the flasks containing MSM and 100ppm of spiromesifen. The experiment was carried out in triplicate and MSM along with spiromesifen flasks without strain SFN1 was treated as control sample. The experiment no 5 with factors temperature 35°C, pH 7, and inoculum size 2.5 (OD @ 600nm) resulted in the highest degradation with a value of 83.210% as well as S/N ratio (38.4035) while minimum degradation (76.640%) and S/N ratio (37.6891) were observed in experiment No 1 (Table 4.39). The optimum parameters for degrading spiromesifen were selected on the basis of Signal to Noise ratio by selecting the option 'larger is better' value. Based on the above test results, the Delta ranking of the parameter is that temperature influences the spiromesifen removal rate the most, followed by pH, and inoculum size (A>B>C). On the basis of main effect plots, the optimum parameters suggested by Taguchi were temperature 35°C, pH was 7 and inoculum size was 2 (OD @600nm) ($A_2B_2C_2$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 d) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that has maximum effect on the biodegradation of spiromesifen by strain SFN1 was temperature while inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 85.92% (S/N – 38.70) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 35°C (Level 2), pH 7 (Level 2), and inoculum density 2 (Level 2). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the

optimum response, using predicted optimum conditions. Observed quinalphos degradation was 86.3 % which is significantly similar to the predicted value (85.92%) at 95% level of confidence.

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	Inoculum size
1	37.87	38.02	38.07
2	38.33	38.33	38.25
3	38.12	37.97	38.01
Delta	0.46	0.36	0.25
Rank	1	2	3

Response Table for Means

Level	Temperature	pH	Inoculum size
1	78.34	79.67	80.07
2	82.54	82.54	81.80
3	80.56	79.23	79.56
Delta	4.20	3.31	2.24
Rank	1	2	3

Figure 4.33: Response table for means and signal to noise ratio of Spiromesifen degradation

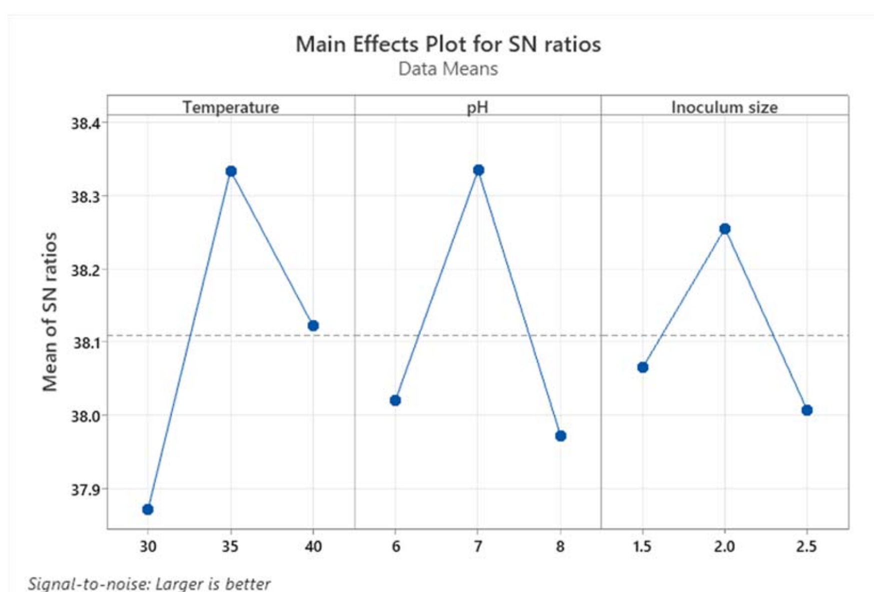


Figure 4.34: Main effect plot of SN ratios of parameters for Spiromesifen degradation

Mate et al., (2015) studied the photo-degradation of spiromesifen in agricultural fields. They reported that spiromesifen is more susceptible to photodegradation and that all environmental conditions affect this degradation. Additionally, they found that the degradation of spiromesifen in soil follows first order kinetics. In this study, the kinetic modelling of spiromesifen degradation by strain SFN1 was done, and it was found that it follows first-order kinetics (Table 4.39), which is in agreement with the previous study report. Studies on the biodegradation of spiromesifen are limited. The only available study was of Manimozhi et al., (2022), where they isolated spiromesifen degrading bacterium *Serratia sp*. from different ecosystems. They reported that *Serratia sp*. degraded about 5000ppm of spiromesifen in MSM after 10

days of incubation. Whereas, in this study, strain SFN1 degraded 100ppm of spiromesifen in MSM with a removal rate of 83.21% after 10 days of incubation. It is important to note that the present study is the first report of degradation of spiromesifen by *Stenotrophomonas [Pseudomonas] geniculata* strain SFN1.

The degradation potential of *Stenotrophomonas [Pseudomonas] geniculata* is roughly explored by researchers. Liu et al., (2014) isolated *P. geniculata* strain N1 from tobacco leaves with the potential to degrade nicotine. They reported that the maximum cell growth and nicotine degradation were obtained at the temperature 30°C and pH between 5.5 to 7.5. No growth was observed at the temperature 35°C. This is slightly different from the findings of this study because strain SFN1 exhibited maximum growth rate and degradation between temperatures 30°C to 40°C. This difference can be due to the isolation of bacterial species one from soil and strain N1 from tobacco leaves. Wu et al., (2020) reported that *P.geniculata* strain PQ01 facilitates the breakdown of commonly used pesticide paraquat. This strain PQ01 reduced paraquat from 50 mg/L to 16 mg/L in 12 days in the presence of a second carbon source sucrose in the media. They also examined the optimum growth conditions of *P.geniculata*. According to their report, *P geniculata* exhibits maximum growth at temperatures between 25 to 37°C, and no growth below 5°C and above 45°C. It exhibited maximum growth at pH 6 to 9, and no growth at pH 4. Strain SFN1 demonstrated a removal rate of 82.31% of spiromesifen in 10 days after incubation. It exhibited maximum growth at temperatures between 25 to 40°C and minimum growth at 20°C. The maximum growth of strain SFN1 was observed at pH between 6 to 8 and minimum growth at pH 5. These findings align with those of Wu et al., (2020), in the inference of growth conditions of *P.geniculata.* Gopalakrishnan et al., (2015) isolated three bacterial species from the root nodules of plants including *P.geniculata* with the ability to degrade pesticides. Researchers also explored the other functional characteristics of *P.geniculata*, such as active in nitrogen fixation (Zhang et al., 2010), promotes plant growth (Gopalakrishnan et al., 2015), and promotes the growth of seedlings from old seeds (Liu et al., 2019). Significantly, the present study is the first report to demonstrate the spiromesifen degrading potential of strain SFN1.

4.4.1.5 Degradation of Fenpyroximate by *Paenibacillus alvei* strain F1T

The degradation of fenpyroximate by *Paenibacillus alvei* strain F1T was analysed at different temperatures (15°C to 40°C), at different pH (5 to 9), and by applying different bacterial inoculum size (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain F1T was also analysed at different temperatures and pH. The fenpyroximate was degraded at all the selected temperatures at different rates. The maximum degradation mean value of 81.006 ± 0.005% was observed at 35°C. Degradation mean value of 80.5 ± 0.1% and 79.07±0.01% was observed at 30°C and 40°C respectively. The low degradation of fenpyroximate was observed at temperatures 15°C and 20°C with the mean values of 8.6 ±0.01% and 21.76 ± 0.05% respectively. In the analysis based on different pH, the maximum degradation was observed at pH 7 with a mean value of 81.01 ± 0.01%. The degradation with a mean value of 79.05± 0.01% and 68.366 ± 0.152% was observed at pH 6 and pH 5 respectively. This could be attributed to the fact that strain F1T prefers neutral pH for the metabolization of fenpyroximate. When different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 2.5 (OD @ 600nm) with a mean value of 80.99 ± 0.005%. This could be because the rate of fenpyroximate degradation increases with an increase in bacterial growth. A degradation of fenpyroximate with a mean value of 80.66± 0.05% and 79.66 ±0.05% was attained by the addition of inoculum size 2 and 1.5 (OD@600nm). It was noted that strain F1T adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C). Its growth is only low at 15°C and 20°C with a mean value of 0.218± 0.02 and 0.306± 0.005 respectively. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 2.02 ± 0.01. The strain F1T can grow in the pH range of 5 to 9. The highest OD value of 2.02±0.02 was observed at pH 7 and the lowest at pH 9 with a value of 0.616 ± 0.01. The degradation rate of fenpyroximate in the control flasks (MSM and fenpyroximate, without the strain F1T) was about 19.2 ± 0.02% after 10 days of incubation.

To optimise fenpyroximate degradation by strain F1T, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell

concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L 9[3]^{3}) was designed with the following factors: temperature (A-30, 35, 40°C), solution pH (B – 5.0, 6.0, 7.0), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm). The strain F1T was inoculated into the flasks containing MSM and 100ppm of fenpyroximate. The experiment was carried out in triplicate and MSM along with fenpyroximate flasks without strain F1T was treated as the control sample. The experiment no 3 with factors temperature 30°C, pH 7, and inoculum size 2.5 (OD @ 600nm) resulted in the highest degradation with a value of 80.99% as well as S/N ratio (37.89) while minimum degradation (76.01%) and S/N ratio (37.65) were observed in experiment No 7 (Table 4.36). The optimum parameters for degrading fenpyroximate were selected on the basis of Signal to Noise ratio by selecting the option 'larger is better' value. Based on the above test results the Delta ranking of the parameter is that pH influences the fenpyroximate removal rate the most, followed by temperature, and inoculum size (B>A>C). On the basis of the main effect plots, the optimum parameters suggested by Taguchi were temperature 35°C, pH was 7, and inoculum size was 2.5 (OD @600nm) ($A_2B_3C_3$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 j) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that pH has maximum effect on the biodegradation of fenpyroximate by strain F1T while inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 80.99% (S/N – 38.169) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 35oC (Level 2), pH 7 (Level 3), and inoculum density 2.5 (Level 3). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed fenpyroximate degradation was 81.2 % which is significantly similar to the predicted value (80.99%) at 95% level of confidence.

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	Inoculum size
1	37.95	37.65	37.91
2	37.98	38.05	37.95
3	37.89	38.12	37.95
Delta	0.08	0.46	0.04
Rank	2	1	3

Response Table for Means

Level	Temperature	pH	Inoculum size
1	78.97	76.32	78.66
2	79.23	79.88	79.01
3	78.49	80.50	79.03
Delta	0.74	4.19	0.38
Rank	2	1	3

Figure 4.35: Response tables for SN ratios and Means of Fenpyroximate degradation by F1T

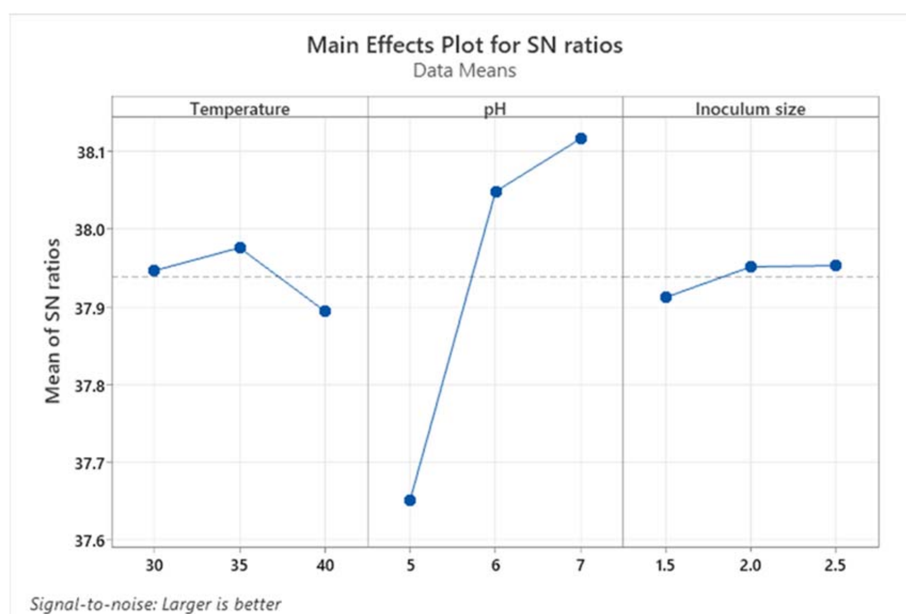


Figure 4.36: Main effects plot for SN ratios of parameter optimization of fenpyroximate by F1T.

A review of previous research indicated that no literature is available on the degradation of fenpyroximate by soil bacteria and the present study is the first to report the degradation of fenpyroximate by *Paenibacillus alvei* strain F1T. However, the degradation potential of the bacteria *Paenibacillus sp*. has been explored by researchers. Daane et al., (2001) isolated *Paenibacillus sp*. from the rhizosphere of salt marsh plants and reported the degradation ability of this strain towards Polycyclic Aromatic Hydrocarbon (PAH). Birolli et al., (2020) isolated bacterial sp. from orange cultivation plots and formulated a microbial consortium, with five bacterial strains including *Paenibacillus alvei* CBMAI2221 for the degradation of fungicide pyraclostrobin. They reported that the combined action of both *Bacillus sp.* CSA-13 and *Paenibacillus alvei* CBMAI2221 increased the removal rate of pyraclostrobin with a degradation value of 25 percent. Jimoh and Lin, (2022) studied the degradation

of n hexadecane and diesel fuel by *Paenibacillus sp*. The strain degraded them with a value of 98.4% and 61.2 % respectively after 14 days of incubation. They also reported the biosurfactant property of *Paenibacillus sp.* which enhanced the rate of biodegradation of the two compounds. All these studies imply that *Paenibacillus sp*. is highly able to degrade several xenobiotic compounds from the environment.

4.4.1.6 Degradation of Quinalphos by *Paenibacillus alvei* strain Q1T

The degradation of quinalphos by *Paenibacillus alvei* strain Q1T was analysed at different temperatures (15°C to 40°C), at different pHs (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain Q1T was also analysed at different temperatures and pHs. The quinalphos were degraded at all the selected temperatures at different rates. The maximum degradation mean value of 62.53 ± 0.37% was observed at 35°C. Degradation mean value of 60.13 ± 0.15% and 56.42±0.03% was observed at 40°C and 30°C respectively. The low degradation of quinalphos was observed at temperatures 15°C and 20°C with mean values of 12.8±0.06% and 20.08 ± 0.08% respectively. In the analysis based on different pH, the maximum degradation was observed at pH 7 with a mean value of 69.18 ± 0.07%. The degradation with a mean value of 59.61± 0.03% and 48.59 ± 0.07% was observed at pH 6 and pH 5 respectively. This could be attributed to the fact that strain Q1T prefers neutral pH for the metabolization of quinalphos. When a different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 2 (OD @ 600nm) with a mean value of 60.65 ± 0.13%. This could be because the rate of quinalphos degradation increases with an increase in bacterial growth. A degradation of quinalphos with a mean value of 51.36± 0.21% and 49.53 ±0.3% was attained by the addition of inoculum sizes 1.5 and 2.5 (OD@600nm). It was noted that strain Q1T adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C). Its growth is only low at 15°C and 20°C with a mean value of 0.225± 0.01 and 0.501± 0.01 respectively. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 2.01 ± 0.01. The strain Q1T can grow in the pH range of 5 to 9. The highest OD value of 2.1±0.10 was observed at pH 7 and the lowest at pH 9 with a value of 0.12 ± 0.01. The degradation rate of quinalphos in the control flasks (MSM and quinalphos, without the strain Q1T) was about 16.5 ± 0.02% after 10 days of incubation. In the

initial phase, the degradation rate and bacterial growth were very slow ; after two days of incubation the degradation rate increased gradually and attained a maximum level within five to eight days.

To optimise quinalphos degradation by strain Q1T, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L $9[3]^{3}$) was designed with the following factors: temperature (A-30, 35, 40°C), solution pH (B – 5.0, 6.0, 7.0), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm)(Figure 4.37). The strain Q1T was inoculated into the flasks containing MSM and 100ppm of quinalphos. The experiment was carried out in triplicate and MSM along with quinalphos flasks without strain Q1T was treated as a control sample. The experiment no 9 with factors temperature 40°C, pH 7, and inoculum size 2 (OD @ 600nm) resulted in the highest degradation with a value of 63.198% as well as S/N ratio (36.0141) while minimum degradation (52.123%) and S/N ratio (34.3406) were observed in experiment No 1 (Table 4.44). The optimum parameters for degrading quinalphos were selected based on Signal to Noise ratio by selecting the option 'larger is better' value. Based on the above test results the Delta ranking of the parameter is that pH influences the quinalphos removal rate the most, followed by temperature, and inoculum size (B>A>C). On the basis of main effect plots, the optimum parameters suggested by the Taguchi OA method were temperature 35°C, pH was 7 and inoculum size was 2 (OD @600nm) ($A_2B_3C_2$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 i) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that pH has maximum effect on the biodegradation of quinalphos while inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 64.46% (S/N – 36.23) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 35°C (Level 2), pH 7 (Level 3), and inoculum density 2.5 (Level 2). An experiment was conducted to confirm the

fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed quinalphos degradation was 64.39 % which is significantly similar to the predicted value (64.46%) at 95% level of confidence.

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	Inoculum size
1	35.02	34.65	35.06
2	35.53	35.42	35.53
3	35.20	35.68	35.17
Delta	0.51	1.02	0.47
Rank	2	1	3

Response Table for Means

Level	Temperature	pH	Inoculum size
1	56.46	54.09	56.71
2	59.79	59.02	59.81
3	57.70	60.84	57.43
Delta	3.33	6.75	3.10
Rank	2	1	3

Figure 4.37: Response tables of SN ratios and means of Quinalphos degradation by Q1T

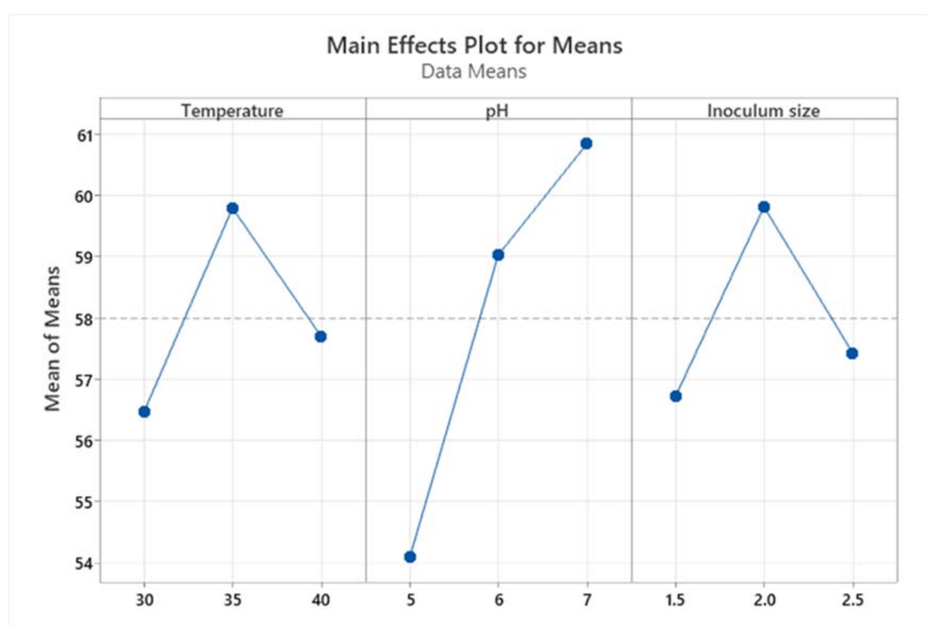


Figure 4.38: Main effects plot for means of Quinalphos degradation by Q1T

The degradation of quinalphos in the environment naturally occurs through the hydrolysis of P-O alkyl and P-O aryl bonds (Dhanjal et al., 2014). In the present study, quinalphos was degraded by strain Q1T with a value of 63.18% after 10 days of incubation. Similarly, Dhanjal et al., (2014) reported that 20ppm of quinalphos was degraded by the combined effect of *Bacillus sp*. and *Pseudomonas sp*. with values of 87% and 80% respectively within a short span of time of 17 days in liquid media. They isolated the bacterial sp. from soil samples from Punjab. They recognised that the increase in bacterial growth enhances the rate of biodegradation, when the transition phase of growth is attained by the microorganisms the energy sources will be metabolised at their maximum level. Throughout the study, the high inoculum

size increased the rate of biodegradation and decreased the half-life of the pesticides. Only quinalphos was added as the only energy source for the strain Q1T, and it metabolized the quinalphos up to a maximum level. However, Pawar and Mali, (2014) isolated *Pseudomonas* sp. from grape rhizosphere soil and it degraded quinalphos to 90.4% in the presence of additional carbon source glucose. In the absence of glucose, the quinalphos degradation rate was 38.2%. Subsequently, the strain Q1T of this study is one of the best preferences for the degradation of quinalphos. Talwar et al., (2014) demonstrated the degradation of quinalphos by *Ochrobactrum* sp. strain HZM isolated from soil samples. It degraded 2mmol/L of quinalphos with a value of 84.16% at 27°C and pH 7. In the present study also, the maximum degradation was attained at pH 7. The combined effects of bacteria species on the degradation of quinalphos were studied by researchers. Nair et al., (2015) formulated a consortium consisting of *P.aeruginosa, Serratia sp* and *Pseudomonas* sp. for the degradation of quinalphos. They observed a degradation value of 85.6% to 93.87% after 8 days of incubation at pH 7 to 8 and temperature 30°C to 37°C. They also explored the biosurfactant properties of the bacterial strains. In comparison to the above study, the maximum degradation was also observed at temperatures of 30°C to 40°C. All the above quoted reports including the findings of the present study reveal that indigenous microorganisms are the finest preference for the biodegradation quinalphos.

4.4.1.7 Degradation of Glyphosate by *Acinetobacter baumannii* strain GLYB2

The degradation of glyphosate by *Acinetobacter baumannii* strain GLYB2 was analysed at different temperatures (15°C to 40°C), at different pHs (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain GLYB2 was also analysed at different temperatures and pHs. The glyphosate was degraded at all the selected temperatures at different rates. Degradation of glyphosate with 32.5%, 55.1%, 60%, 79.05%, 76.15% and 80.016% was observed in the MSM with *Acinetobacter sp*. grown at temperatures of 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. The maximum degradation mean value of 80.016 ± 0.37% was observed at 35°C. Degradation mean value of 60 ± 0.03% and 79.05±0.10% was observed at 40°C and 30°C respectively. The comparatively low degradation of glyphosate was observed at temperatures 15°C and 20°C with the mean values of 32.5±0.11% and 55.1 ± 0.12% respectively. Growth of strain GLYB2 at pH 5, 6, 7, 8 and 9 caused glyphosate

degradation to the extent of 66.4%, 82.3%, 81.71%, 76.04% and 51.19% % at the end of 10 days of incubation, respectively. The analysis was based on different pH, the maximum degradation was observed at pH 6 with a mean value of 82.3 ± 0.04%. The degradation with a mean value of 81.71± 0.04% and 76.04 ± 0.08% was observed at pH 7 and pH 8 respectively. This could be attributed to the reason that strain GLYB2 prefers slightly acidic pH for the metabolization of glyphosate. When different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 2 (OD @ 600nm) with a mean value of 83.02 ± 0.03%. This could be because the rate of glyphosate degradation increases with an increase in bacterial growth. A degradation of glyphosate with a mean value of 81.18± 0.15% and 76.02 ±0.32% was attained by the addition of inoculum sizes 1.5 and 2.5 (OD@600nm). It was observed that the strain GLYB2 adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C) to a maximum level. Its growth is only low at 15°C and 20°C with a mean value of 1.2± 0.06 and 1.7 ± 0.043 respectively when compared to other selected temperature ranges. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 2.9 ± 0.06. The strain GLYB2 can grow in the pH range of 5 to 9. The highest OD value of 2.73 ± 0.02 was observed at the pH 6 and the lowest at the pH 9 with a value of 1.3 ± 0.01. The degradation rate of glyphosate in the control flasks (MSM and glyphosate, without the strain GLYB2) was about 27.6 ± 0.21% after 10 days of incubation. In the degradation of glyphosate by GLYB2, it exhibited almost an acceptable level of removal rate of glyphosate at all the parameters selected for the study.

To optimise glyphosate degradation by strain GLYB2, the single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test ($L\ 9[3]^3$) was designed with the following factors: temperature (A- 25, 30, 35°C), solution pH (B – 6.0, 7.0, 8), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm) (Figure 4.39). The strain GLYB2 was inoculated into the flasks containing MSM and 100 ppm of

glyphosate. The experiment was carried out in triplicate and MSM along with glyphosate flasks without strain GLYB2 was treated as a control sample. Experiment no 9 with factors temperature 35°C, pH 8, and inoculum size 2 (OD @ 600nm) resulted in the highest degradation with a value of 83.020% as well as S/N ratio (38.3837) while minimum degradation (74.042%) and S/N ratio (37.3896) were observed in experiment No 8 (Table 4.41). The optimum parameters for degrading glyphosate were selected on the basis of Signal to Noise ratio by selecting the option 'larger is better' value. Based on the above test results, the Delta ranking of the parameter is that inoculum size influences the glyphosate removal rate the most, followed by pH, and temperature (C>B>A). Based on the main effect plots, the optimum parameters suggested by the Taguchi OA method were temperature 35°C, pH was 6 and inoculum size was 2 (OD @600nm) ($A_3B_1C_2$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table4.46 e) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that inoculum density has maximum effect on the biodegradation of glyphosate while pH was the least contributing factor. As predicted by the Taguchi DOE, 84.20% (S/N – 39.5149) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 35°C (Level 3), pH 6 (Level 1), and inoculum density 2 (Level 2). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed propargite degradation was 84.35 % which is significantly similar to the predicted value (84.20%) at 95% level of confidence.

Response Table for Means

Level	Temperature	pH	inoculum size
1	78.59	81.21	77.30
2	79.10	77.44	81.50
3	79.85	78.90	78.74
Delta	1.26	3.78	4.20
Rank	3	2	1

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	inoculum size
1	37.90	38.19	37.76
2	37.96	37.77	38.22
3	38.03	37.93	37.92
Delta	0.13	0.42	0.46
Rank	3	2	1

Figure 4.39: Response table for means for glyphosate degradation.

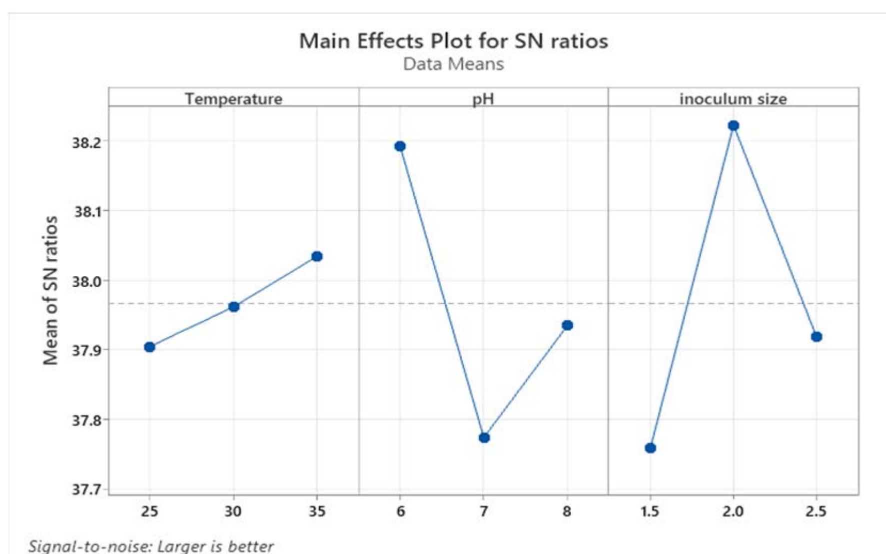


Figure 4.40: Main effects plots for glyphosate by *Acinetobacter baumannii GLYB2*

Previous investigations have documented the efficacy and potential of microorganism s for rapid and easy bioremediation of glyphosate-contaminated areas. Glyphosate degrading microorganisms *Flavobacterium sp*. (Balthazor and Hallas, 1986), *Pseudomonas sp*. (Kishore and Jacob, 1987), *Arthrobacter sp.* GLP-1 (Pipke et al., 1987), *Arthrobacter atrocyaneus* ATCC (Pipke and Amrhein, 1988), *Pseudomonas* strain LBr (Jacob et al., 1988) had been previously isolated from contaminated sites by enrichment culture techniques. Therefore, it is abundantly clear from the above mentioned studies that a range of indigenous bacteria are capable of successfully degrading glyphosate. Fan et al., (2012) examined the degradation of glyphosate by *Bacillus cereus* strain CB4. They also reported the optimum conditions like 35°C, pH 6, substrate concentration 6g/L, and inoculum amount 5% for maximum degradation of glyphosate with a value of 94.47%. The strain *B. cereus* CB4 degraded 12g/L of glyphosate after 5 days of incubation. In the present study the maximum degradation was attained at pH 8 which is alkaline in nature and the optimum pH suggested by Taguchi OA method was pH 6. Yu et al., (2015) reported that the glyphosate degradation by *B. subtilis* BS-15 was maximum with a value of 66.97% within 96 hours at 35°C and pH 8. This report aligns with the findings of the present study. However, Fan et al., (2012) reported that the maximum degradation of glyphosate by *B. cereus* strain CB4 was observed at pH 6, and the degradation was inhibited under acidic conditions (pH 2 to 5) and alkaline conditions (pH 8 to 12). This could be attributed to the difference in bacterial species and their preference for diverse environmental conditions for growth. In this study the kinetic modelling of

glyphosate degradation was studied and it was observed that the degradation of glyphosate follows first order kinetics (Table 4.41) with regression values greater than 0.94. A comparable finding was observed by Zhao et al., (2016) that the degradation of glyphosate by *Pseudomonas sp*. strains followed the first order model with regression values greater than 0.96. Benslama and Boulahrouf, (2013) isolated four different bacterial species *Pseudomonas putida, E. cloacae, R. aquatilis,* and *S. marcescens* which tolerated up to 9g/L of glyphosate within a short span of time. Kryuchkova et al., (2014) isolated rhizosphere strain *Enterobacter cloacae* K7 which degrades 40% of 10mM glyphosate after 5 days of incubation. In this study, the strain GLYB2 also degraded glyphosate in a liquid medium within 10 days after incubation, which is in agreement with the previously explained results. Benslama and Boulahrouf, (2013) isolated *Enterobacter sp.* strain Bisph2 with the ability to degrade glyphosate of 500mg/L at 30°C. Sabullah et al., (2016) obtained glyphosate degrading indigenous *Klebsiella oxytoca* strain SAW-5 from soil. An indigenous bacterial strain *Providencia rettergi* GDB1 degraded 71.4% of 5000mg/L to 10,000mg/L of glyphosate after 24 hours of incubation (Xu *et al*., 2019). Zhang et al., (2022) isolated *Chryseobacterium sp.* Y16C which degraded both 400mg/L of glyphosate and 800mg/L of AMPA (metabolite of glyphosate) within four days of incubation. The observations of the above previous research affirms the findings of this study that the rate of degradation of glyphosate in liquid media varies according to the difference in bacterial species and environmental parameters. It was reported previously *Acinetobacter sp*. could degrade permethrin (Zhan *et al*., 2018), deltamethrin (Tang *et al*., 2020). The present study results recommend that *Acinetobacter baumannii* strain GLYB2 can be used for the efficient degradation of glyphosate and other xenobiotic compounds.

4.4.1.8 Degradation of Propargite by *Chryseobacterium cucumeris* strain PTEB2

The degradation of propargite by *Chryseobacterium cucumeris* strain PTEB2 was analysed at different temperatures (15°C to 40°C), at different pHs (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain PTEB2 was also analysed at different temperatures and pHs. The propargite was degraded at all the selected temperatures at different rates. Degradation of glyphosate with 33.45%, 41.43%, 66.73%, 70.41%, 72.58% and 30.13% was observed in the MSM with

Chryseobacterium cucumeris PTEB2 grown at temperatures of 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. The maximum degradation mean value of 72.58 ± 0.50% was observed at 35°C. Degradation mean value of 70.41 ± 0.35% and 66.73±0.64% was observed at 30°C and 25°C respectively. The low degradation of propargite was observed at temperatures 40°C and 15°C with the mean values of 30.13±0.11% and 33.45 ± 0.11% respectively. Growth of strain PTEB2 at pH 5, 6, 7, 8 and 9 caused propargite degradation to the extent of 32.14%, 73.51%, 71.15%, 70.51%, and 50.38% % at the end of 10 days of incubation, respectively. The analysis was based on different pH, the maximum degradation was observed at pH 6 with a mean value of 73.51 ± 0.02%. The degradation with a mean value of 71.15± 0.13% and 70.51 ± 0.46% was observed at pH 7 and pH 8 respectively. This could be attributed to the reason that strain PTEB2 prefers slightly acidic to neutral pH for the metabolization of propargite. When different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 1.5 (OD @ 600nm) with a mean value of 74.15 ± 0.13%. This could be due to the reason that the rate of propargite degradation increases with an increase in bacterial growth. A degradation of propargite with a mean value of 73.77± 0.15% and 70.09 ±0.09% was attained by the addition of inoculum sizes 2 and 1 (OD@600nm). It was observed that strain PTEB2 adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C) to a maximum level. Its growth is only low at 15°C and 40°C with a mean value of 0.71± 0.01 and 0.62 ± 0.11 respectively when compared to other selected temperature ranges. In the range of 15 to 40°C the culture value was highest at temperature 35°C with a mean value of 1.95 ± 0.04. The strain PTEB2 can grow in the pH range of 5 to 9. The highest OD value of 1.9 ± 0.01 was observed at pH 6 and the lowest at pH 5 with a value of 0.636 ± 0.015. The degradation rate of propargite in the control flasks (MSM and propargite, without the strain PTEB2) was within the range of 21.5 ± 0.01% to 22.6 ± 0.25% after 10 days of incubation.

Response Table for Signal to Noise Ratios

Larger is better

Level	temperature	pH	Inoculum size
1	37.09	37.15	37.04
2	36.98	37.13	37.24
3	37.24	37.04	37.04
Delta	0.26	0.11	0.20
Rank	1	3	2

Response Table for Means

Level	temperature	pH	Inoculum size
1	71.58	72.00	71.12
2	70.61	71.84	72.75
3	72.79	71.14	71.11
Delta	2.19	0.86	1.65
Rank	1	3	2

Figure 4.41: Response table for S/N ratios of the parameters.

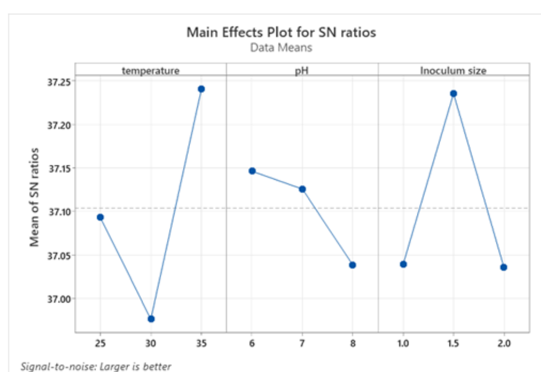


Figure 4.42: Main effects plots for glyphosate by *Chryseobacterium cucumeris* PTEB2

To optimise propargite degradation by strain PTEB2, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L $9[3]^3$) was designed with the following factors: temperature (A- 25, 30, 35°C), solution pH (B – 6.0, 7.0, 8), and inoculum size (C – 1,1.5, 2.0 OD @ 600nm) (Figure 4.42). The strain PTEB2 was inoculated into the flasks containing MSM and 100ppm of propargite. The experiment was carried out in triplicate and MSM along with propargite flasks without strain PTEB2 was treated as a control sample. Experiment no 9 with factors temperature 35°C, pH 8, and inoculum size 1.5 (OD @ 600nm) resulted in the highest degradation with a value of 74.150% as well as S/N ratio (37.4022) while minimum degradation (70.050%) and S/N ratio (36.9082) were observed in experiment No 6 (Table 4.40). The optimum parameters for degrading propargite were selected on the

basis of Signal to Noise ratio by selecting the option ‘larger is better’ value. Based on the above test results the Delta ranking of the parameter is that temperature influences the propargite removal rate the most, followed by inoculum size and pH (A>C>B). On the basis of main effect plots, the optimum parameters suggested by the Taguchi OA method were temperature 35°C, pH was 6 and inoculum size was 1.5 (OD @600nm) ($A_3B_1C_2$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 f) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that temperature has maximum effect on the biodegradation of propargite while pH was the least contributing factor. As predicted by the Taguchi DOE, 74.22% (S/N – 37.4147) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 35°C (Level 3), pH 6 (Level 1), and inoculum density 1.5 (Level 2). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed propargite degradation was 74 % which is significantly similar to the predicted value (74.22%) at 95% level of confidence.

A similar study at the same ecosystem was done by Sarkar et al., (2010). studied the degradation of propargite by *Pseudomonas putida* isolated from the tea garden rhizosphere soils. They reported that *Pseudomonas sp.* degraded propargite with a value of 69% and 71.9% in 24 hours in the presence of glucose in the MSM. This is the only available literature report of propargite degradation by soil bacteria until now. In the present study, *C.cucumeris* strain PTEB2 degraded propargite with a value of 74.15% after 10 days of incubation without the presence of any additional carbon source. *Chryseobacterium* sp. has been explored to treat various environmental pollutants such as the organochlorine pesticides –flubendiamide (Jadhav et al., 2016), oxyfluorfen (Zhao et al., 2016), carbendazim (Silambarasan and Abraham, 2020), and glyphosate (Zhang et al., 2022). However, there are no reports of strains in the genus *Chryseobacterium* for degradation of propargite to date. The present study is the first report on the degradation of propargite by *Chryseobacterium cucumeris* strain PTEB2. This study further indicates that the optimum conditions for the maximum degradation of propargite by *C.cucumeris* strain PTEB2.

4.4.1.9 Degradation of spiromesifen by *Bacillus subtilis* strain SFT1

The degradation of spiromesifen by *Bacillus subtilis* strain SFT1 was analysed at different temperatures (15°C to 40°C), at different pHs (5 to 9), and by applying different bacterial inoculum sizes (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain SFT1 was also analysed at different temperatures and pH. The spiromesifen was degraded at all the selected temperatures at different rates. Degradation of spiromesifen with 26.11%, 48.15%, 52.03%, 65.00%, 71.65%, and 72.89% was observed in the MSM with *Bacillus subtilis* SFT1 grown at temperatures of 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. The maximum degradation mean value of 72.89 ± 0.03% was observed at 30°C. Degradation mean value of 71.65 ± 0.03% and 65.00±0.02% was observed at 35°C and 40°C respectively. The low degradation of spiromesifen was observed at temperature 20°C and 15°C with mean values of 48.15±0.05% and 26.11 ± 0.02% respectively. Growth of strain SFT1 at pH 5, 6, 7, 8 and 9 caused spiromesifen degradation to the extent of 32.16%, 72.88%, 69.99%, 69.40%,, and 51.07 % at the end of 10 days of incubation, respectively. The analysis based on different pH, the maximum degradation was observed at pH 6 with a mean value of 72.88 ± 0.03%. The degradation with a mean value of 69.99± 0.03% and 69.40 ± 0.39% was observed at pH 7 and pH 8 respectively. This could be attributed to the reason that strain SFT1 prefers slightly acidic to neutral pH for the metabolization of spiromesifen. When a different inoculum size was introduced to the media, the maximum degradation observed was at the inoculum size 2 (OD @ 600nm) with a mean value of 73.02 ± 0.03%. This could be due to the reason that the rate of spiromesifen degradation increases with an increase in bacterial growth. A degradation of spiromesifen with a mean value of 72.62± 0.02% and 69.76 ±0.14% was attained by the addition of inoculum sizes 1.5 and 2.5 (OD@600nm). It was noted that strain SFT1 adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C) to a maximum level. Its growth is only low at 15°C and 40°C with a mean value of 0.62± 0.01 and 1.01 ± 0.02 respectively when compared to other selected temperature ranges. In the range of 15 to 40°C the culture value was highest at temperature 30°C with a mean value of 1.80 ± 0.01. The strain SFT1 can grow in the pH range of 5 to 9. The highest OD value of 1.84 ± 0.02 was observed at the pH 6 and the lowest at the pH 5 with a value of 0.95 ± 0.04. The degradation rate of

spiromesifen in the control flasks (MSM and spiromesifen, without the strain SFT1) was within the range of 18.9 ± 0.05% to 20.1 ± 0.02% after 10 days of incubation.

Response Table for Means

Level	Temperature	pH	inoculum sze
1	72.56	71.99	69.76
2	71.70	69.13	71.35
3	67.84	70.98	70.98
Delta	4.72	2.86	1.59
Rank	1	2	3

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	inoculum sze
1	37.21	37.14	36.86
2	37.11	36.79	37.06
3	36.63	37.02	37.02
Delta	0.59	0.36	0.20
Rank	1	2	3

Figure 4.43: Response tables for means of Spiromesifen By *Bacillus subtillis SFT1*

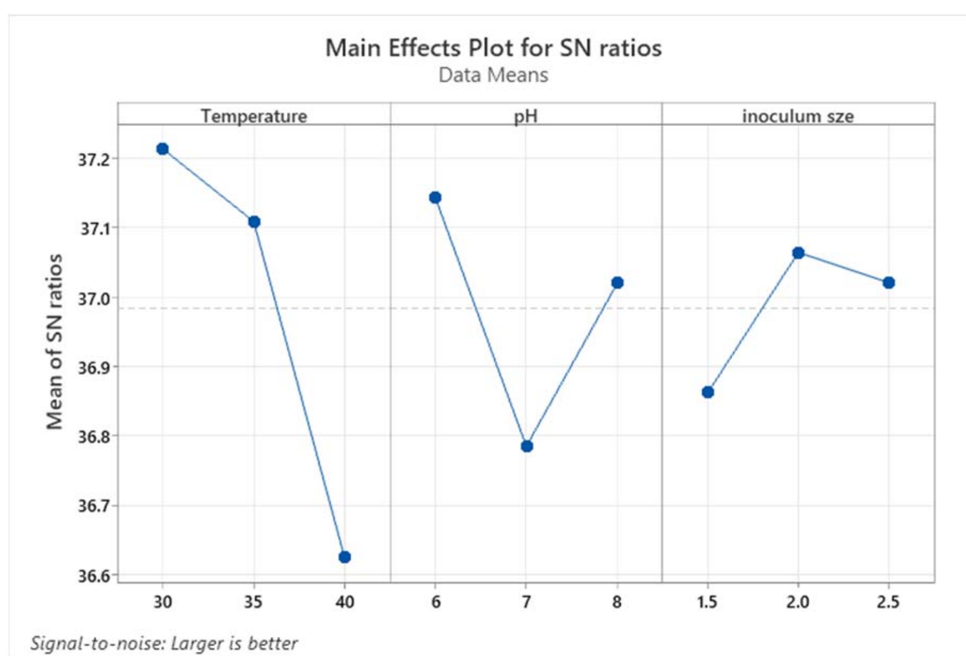


Figure 4.44: Main effects plot for SN ratios of *Bacillus subtilis SFT1*

To optimise spiromesifen degradation by strain SFT1, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L $9[3]^3$) was designed with the following factors: temperature (A- 30, 35, 40°C), solution pH (B – 6.0, 7.0, 8), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm) (Figure 4.43). The strain SFT1

was inoculated into the flasks containing MSM and 100ppm of spiromesifen. The experiment was carried out in triplicate and MSM along with spiromesifen flasks without strain SFT1 was treated as a control sample. Experiment no 4 with factors temperature 35°C, pH 6, and inoculum size 2 (OD @ 600nm) resulted in the highest degradation with a value of 73.52% as well as S/N ratio (37.3281) while minimum degradation (65.21%) and S/N ratio (36.2863) were observed in experiment No 8 (Table 4.38). The optimum parameters for degrading spiromesifen were selected on the basis of Signal to Noise ratio by selecting the option 'larger is better' value. Based on the above test results, the Delta ranking of the parameter is that temperature influences the spiromesifen removal rate the most, followed by pH and inoculum size (A>B>C). Based on main effect plots, the optimum parameters suggested by the Taguchi OA method were temperature 30°C, pH was 6 and inoculum size was 2 (OD @600nm) ($A_1B_1C_2$). Due to the interactions of significant factors and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 c) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that temperature has maximum effect on the biodegradation of spiromesifen by strain SFT1 while inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 74.49% (S/N – 37.4550) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 30°C (Level 1), pH 6 (Level 1), and inoculum density 2 (Level 2). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed spiromesifen degradation was 74.01 % which is significantly similar to the predicted value (74.49%) at 95% level of confidence.

The biodegradation studies of spiromesifen by soil bacteria were quite limited. The only available study was of Manimozhi et al., (2022), where they isolated spiromesifen degrading bacterium *Serratia sp.* from different ecosystems. They reported that *Serratia sp.* degraded about 5000ppm of spiromesifen in MSM after 10 days of incubation. Whereas, in the present study, strain SFT1 degraded 100ppm of spiromesifen in MSM with a removal rate of 73.52% after 10 days of incubation. A survey of previous research in the area indicates that the present study is the first report of degradation of spiromesifen by *Bacillus subtilis* strain SFT1. It also studied

the kinetic modelling of spiromesifen degradation by strain SFT1 and found that it follows first order kinetics with a regression value of 0.94.

It was reported previously that *Bacillus sp*. could degrade different pesticides like nicosulfuron (Lu et al., 2019), chlorpyrifos (El-Helow et al., 2013), profenofos (Salunkhe et al., 2014), carbendazim (Salunkhe et al., 2014), beta-cypermethrin (Xiao et al., 2015), pendimethalin (Ni et al., 2016), cypermethrin (Gangola et al., 2017), and penthiopyrad (Podbielska et al., 2020). All these studies validate the findings of this study that *Bacillus subtilis* is the finest preference for the biodegradation of xenobiotic compounds.

4.4.1.10 Degradation of fenpyroximate by *Pseudomonas aeruginosa* strain FXE1

The degradation of fenpyroximate by *Pseudomonas aeruginosa* strain FXE1 was analysed at different temperatures (15°C to 40°C), at different pHs (5 to 9), and by applying different bacterial inoculum size (0.5 to 2.5 OD @ 600nm). The incubation period was ten days in liquid MSM. The growth of the strain FXE1 was also analysed at different temperatures and pHs. The fenpyroximate was degraded at all the selected temperatures at different rates. Degradation of fenpyroximate with 36.92%, 41.58%, 75.12%, 81.4%, 70.47%, and 51.13% was observed in the MSM with *Pseudomonas aeruginosa* FXE1 grown at temperatures of 15°C, 20°C, 25°C, 30°C, 35°C, and 40°C. The maximum degradation mean value of 81.4 ± 0.2% was observed at 30°C. Degradation mean value of 75.12 ± 0.03% and 70.47±0.31% was observed at 25°C and 35°C respectively. The low degradation of fenpyroximate was observed at temperatures 15°C and 20°C with the mean values of 36.92 ± 0.21% and 41.58 ± 0.05% respectively. Growth of strain FXE1 at pH 5, 6, 7, 8 and 9 caused fenpyroximate degradation to the extent of 56.01%, 66.15%, 79.07%, 71.6%, and 60.05 % at the end of 10 days of incubation, respectively. The analysis was based on different pHs, the maximum degradation was observed at pH 7 with a mean value of 79.07 ± 0.11%. The degradation with a mean value of 71.6± 0.1% and 66.15 ± 0.13% was observed at pH 8 and pH 6 respectively. This could be attributed to the reason that strain FXE1 prefers neutral pH for the metabolization of fenpyroximate. When a different inoculum size was introduced to the media the maximum degradation observed was at the inoculum size 1.5 (OD @ 600nm) with a mean value of 82.07 ± 0.11%. This could be because the rate of fenpyroximate degradation increases with an increase in bacterial growth. A degradation of fenpyroximate with a mean value of

80.3± 0.26% and 72.5 ±0.1% was attained by the addition of inoculum size 2 and 2.5 (OD@600nm). It was noted that strain FXE1 adapts to a wide range of temperatures and exhibits growth at all the selected temperatures (15°C to 40°C) to a maximum level. Its growth is only low at 15°C and 20°C with a mean value of 0.21± 0.11 and 0.45 ± 0.04 respectively. In the range of 15 to 40°C the culture value was highest at temperature 30°C with a mean value of 2.01 ± 0.01. The strain FXE1 can grow in the pH range of 5 to 9. The highest OD value of 1.94 ± 0.02 was observed at the pH 7 and the lowest at the pH 5 with a value of 0.67 ± 0.02. The degradation rate of fenpyroximate in the control flasks (MSM and fenpyroximate, without the strain FXE1) was within the range of 28.75 ± 0.11% to 30.1 ± 0.18% after 10 days of incubation.

To optimise fenpyroximate degradation by strain FXE1, single-factor and orthogonal tests were performed in the laboratory conditions. The single-factor test was designed with the following parameters: temperature 15-40°C, (pH 7.0, and OD 0.5 kept constant), solution pH 5.0-9.0, (temperature 30°C, and OD 0.5 kept constant), and cell concentration OD 0.5 to 2.5, (pH 7.0, and temperature 30°C kept constant). The factors were denoted as temperature – A, pH – B, and inoculum size – C. After analysing the single factor test findings, an orthogonal test (L $9[3]^{3}$) was designed with the following factors: temperature (A- 25, 30, 35°C), solution pH (B – 6.0, 7.0, 8), and inoculum size (C – 1.5, 2.0, 2.5 OD@600nm) (Figure 4.45). The strain FXE1 was inoculated into the flasks containing MSM and 100ppm of fenpyroximate. The experiment was carried out in triplicate and MSM along with fenpyroximate flasks without strain FXE1 was treated as a control sample. Experiment no 6 with factors temperature 30°C, pH 8, and inoculum size 1.5 (OD @ 600nm) resulted in the highest degradation with a value of 78.37% as well as S/N ratio (37.8911) while minimum degradation (69.64%) and S/N ratio (36.8651) were observed in experiment No 7 (Table 4.36). The optimum parameters for degrading fenpyroximate were selected on the basis of Signal to Noise ratio by selecting the option 'larger is better' value. Based on the above test results the Delta ranking of the parameter is that pH influences the fenpyroximate removal rate the most, followed by temperature and inoculum size (B>A>C). On the basis of main effect plots, the optimum parameters suggested by the Taguchi OA method were temperature 30°C, pH was 7 and inoculum size was 1.5 (OD @600nm) ($A_2B_2C_1$). Due to the interactions of significant factors

and their levels in varying combinations maximum biodegradation was achieved. Analysis of variance (Table 4.46 a) depicted that all the selected factors and their interactions in the experimental design were statistically significant at 95% confidence level based on the F-ratio. ANOVA exhibited that pH has maximum effect on the biodegradation of fenpyroximate by strain FXE1 while inoculum density was the least contributing factor. As predicted by the Taguchi DOE, 80.99% (S/N – 38.1925) of degradation can be attained by employing optimised levels of different factors, i.e. temperature 30°C (Level 2), pH 7 (Level 2), and inoculum density 1.5 (Level 1). An experiment was conducted to confirm the fitness of Taguchi Orthogonal array in estimating the optimum response, using predicted optimum conditions. Observed fenpyroximate degradation was 80.12 % which is significantly similar to the predicted value (80.99%) at 95% level of confidence.

Response Table for Signal to Noise Ratios

Larger is better

Level	Temperature	pH	Inoculum size
1	37.52	37.31	37.69
2	37.78	37.81	37.62
3	37.33	37.51	37.32
Delta	0.45	0.51	0.37
Rank	2	1	3

Response Table for Means

Level	Temperature	pH	Inoculum size
1	75.22	73.38	76.67
2	77.41	77.73	76.05
3	73.59	75.11	73.49
Delta	3.83	4.35	3.18
Rank	2	1	3

Figure 4.45: Response tables for Fenpyroximate by *Pseudomonas aeruginosa* FXE1

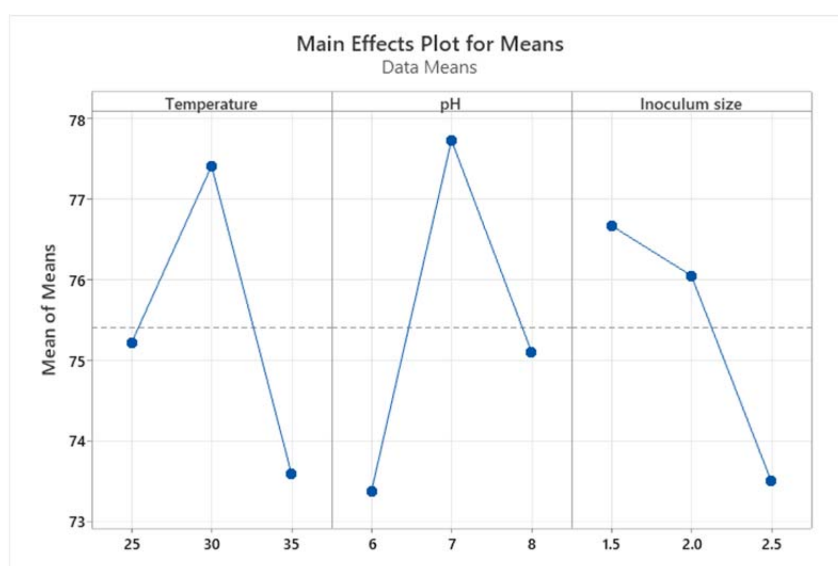


Figure 4.46: Main effects plots for means of *Pseudomonas aeruginosa* FXE1 for fenpyroximate

A survey of research in the area revealed that no studies were reported on the biodegradation of fenpyroximate by soil bacteria. The present study is the first to report on the degradation of fenpyroximate by *Pseudomonas aeruginosa* strain FXE1, with a maximum mean value of 78.37% after 10 days of incubation in liquid MSM. It also demonstrated the optimum environmental conditions for the maximum degradation of fenpyroximate by *Pseudomonas aeruginosa* strain FXE1. Earlier studies have reported that pseudomonas is a resourceful and highly active genus. It was suggested that this genus could degrade a wide range of xenobiotic compounds including pesticides like propiconazole (Sarkar et al., 2009; Satapute and Kaliwal, 2012), acephate (Ramu and Seetharaman, 2019), diazinon (Essa *et al*., 2016), diuron (Kucic *et al*., 2020). All these studies and present findings suggest that *P.aeruginosa* FXE1 could be used effectively for the degradation of fenpyroximate and environmental cleanup of contaminants.

4.4.1.11 Esterase activity of tea plantation soil bacterial isolates

The term "degradative enzymes" refers to microbial enzymes that target specific bonds in the organic pollutant, which are thought to be involved in the microbe-assisted breakdown of pesticides.It is well known that some bacteria have unique enzymes like esterase that may metabolise pesticides (Cycon *et al*., 2009). According to studies (Barone *et al*., 2014; López-López *et al*., 2014; de Lourdes Moreno *et al*., 2016; Parte *et al.,* 2017), esterases are active in bacteria from various genera and play a significant role in the biocatalysis of organic compounds in the food, pharmaceutical, and agrochemical industries, biodiesel production, bioremediation, and waste decontamination. Potentially an effective method for the biodegradation of pesticides is enzymatic bioremediation (Sutherland *et al*., 2004; Bhatt, 2019).

In the present study, all the bacterial strains exhibited esterase activity. The bacterial strains DRNB1, EON2, SFN1 and TXM2showed high esterase activity (Figure 4.47). The strains *Pseudomonas aeruginosa, Chryseobacterium cucumeris* showed decreased esterase activity. The medium-level esterase activity was showed by *Bacillus subtilis*, *Paenibacillus alvei*, and A*cinetobacter baumannii.* According to the nature and kind of substrates, enzymes initiate the important mechanism for substrate degradation (Simon, 2014). Numerous investigations have demonstrated that

carboxylesterase and phosphotriesterase have the ability to hydrolyze a variety of organophosphorus compounds, including esters of phosphoric acid (Simon, 2014).

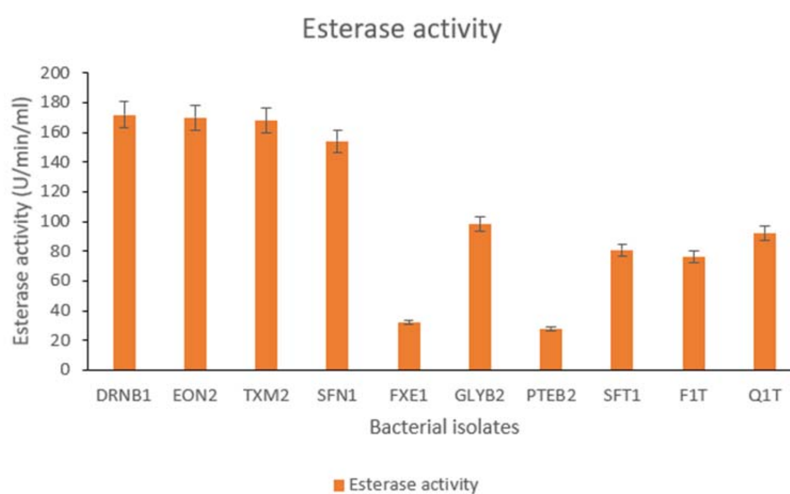


Figure 4.47 Esterases enzyme activity shown by the bacterial isolates

It is generally known that the pyrethroid hydrolase enzyme, also known as pyrethroid catalyzing esterase, is present in a wide range of organisms, including bacteria, fungi, insects, and animals. Esterases are members of the hydrolase enzyme family and are essential regulators in the breakdown of pyrethroids. Microbes, insects, plants, and animal cells have all been found to include pyrethroid-degrading hydrolases in the past (Bai *et al.,* 2019; Wang *et al*., 2018; Yao *et al*., 2018). For the biodegradation of pyrethroids, many strains of bacteria and fungi are utilised, including *Raoultella ornithinolytica*, *Pseudomonas flourescens*, *Brevibacterium spp*., *Acinetobacter* spp., *Aspergillus spp*., *Candida spp*., *Trichoderma spp*., and *Candia spp.* The first step in the biodegradation of pyrethroids is the hydrolysis of the ester link by the enzymes esterase and carboxyl esterase (Bhatt *et al*., 2019). By attacking ester bonds or cytochrome P-450 dependent monooxygenase on acid or alcohol moieties, esterase (carboxyl ester hydrolase) plays a significant part in the early alteration of the parent pyrethroid (Kamita *et al*., 2016). Several pesticides like neonicotinoids, propargite and thiamethoxam degradation studies exhibited esterases activity (Farahani *et al.,*2021). Barraso *et al*.,(2020) studied the esterase activity of *Bradyehizobium* strain in the decomposition of hydrogen peroxide. The present study states that all the bacterial isolates registered esterase activity in different levels.

4.4.2 Degradation studies in soil samples

Degradation of all the selected pesticides with their respective bacterial strains was done in soil samples (500gm). Two sets of soil samples were taken, one is the sterile soil sample and the other sample was sterile but mixed with the particular bacterial strains for the degradation studies (Figure 4.48). The sterile soil samples were kept as the control samples only with pesticides without bacterial cultures. The study was done for a period of three months, i.e.for 90 days. After every 15 days, the pesticide residue was analysed in both the control sterile soil samples and soil samples with bacterial cultures, and the metabolites were detected qualitatively using GC-MS analysis. The moisture content of 40% was maintained, and the incubation temperature was 30±2°C. The inoculum size selected was OD 1.00 @600nm.

Figure 4.48 The degradation of different pesticides in soil samples along with the respective bacterial strains in lab conditions.

4.4.2.1 Degradation of deltamethrin in soil samples by *Stenotrophomonas maltophia* DRNB1

The present study showed that the degradation of deltamethrin in sterilised soil samples inoculated with DRNB1 was observed as 16.5%, 21.8%, 36.5%, 51.2%, 59.6%, and 64.2% during the 15th , 30th , 45th , 60th , 75th and 90th day respectively. In contrast, degradation of deltamethrin control sterilised soil samples without DRNB1 was extremely low during the 90-day experiment time. Nearly 96.8% of the deltamethrin in the control sterilised samples remains intact; only 3.2% of deltamethrin degradation was observed in the control sample. The degradation frequency of deltamethrin in soil augmented with DRNB1 indicated that during the first 30 days, the process was slow. Merely 21.8% of the applied dose of deltamethrin was degraded in soils during 30 days. After 30 days the removal of deltamethrin

increased considerably giving a final value of 64.2% in soils inoculated with DRNB1. The half- life of deltamethrin in soil samples with DRNB1 was determined as 60.73 days. This could be due to the dependency of deltamethrin degradation on the presence of microorganisms. These findings agree with the statements made by Khan et al., (1988), Gu et al., (2008), and Cycon et al., (2014) that microbial degradation is the main process of deltamethrin dissipation in soils. In the present study, it was observed that DRNB1 degraded deltamethrin in both liquid MSM and soil samples. Chen et al., (2011) and Cycon et al., (2014) reported that microorganisms that can degrade synthetic pyrethroids in culture conditions can also disintegrate them in soil. The observed low rate of degradation during the first 30 days might be the result of the necessity of bacterial adaptation to the presence of pesticides, and adaptation to soil conditions. Another reason could be explained by the fact that the soils used in this experiment were forest soils (Virgin soil) which had no contact with any kind of pesticides.Chen et al., (2011), and Cycon et al., (2014) found that the slow rate of deltamethrin dissipation just after its application into soil indicates the time needed for the proliferation of a small population of microorganisms to an optimal level required for an effective degradation of the pesticides. The increased rate of biodegradation and decrease in half-life (60.73 days) in soil inoculated with strain DRNB1 can be explained by the fact that this strain increases the catabolic potential of the soil. In the control samples the removal rate of deltamethrin was only 3.2% and the half-life was 1918.16 days which is approximately 30 times more than in soil samples inoculated with DRNB1. This could be attributed to the presence of microorganisms in the soil that enhances the process of degradation. This fact was also observed in some previous studies. Chen et al., (2011) reported that the inoculation of soil contaminated with fenvalerate (50mg/kg of soil) with *Stenotrophomonas sp*. strain ZS-S-01 increased the degradation rate and decreased the half-life value to eight times lower than for soil without the strain. They also reported that the pyrethroid contaminated (50mg/kg of soil) soil inoculated with beta-cypermethrin degrading *S. aureus* HP-S-01 resulted in a decrease of beta - cypermethrin at a level of 87.8% within 10 days of the experiment, whereas the degradation rate of beta-cypermethrin in non-inoculated samples reached only 25.1% (Chen et al., 2012). Cycon et al., (2014) demonstrated that the half-life of deltamethrin in soils inoculated with the bacterial strain Del-1 and Del-2 decreased to 2.8 days than soils without the bacterial *Serratia sp*. Zhang et al., (2016) reported that

the soil samples inoculated with *Bacillus cereus* strain Y1 degraded 74.9% of 10 mg/kg of deltamethrin in 25 days, whereas only 45.1% of deltamethrin was removed from control samples lacking the strain Y1. Wu et al., (2021) described that *Stenotrophomonas maltophilia* strain XQ08 degraded deltamethrin in soils inoculated with strain XQ08 within range of 93.89% to 96.38% within 7 days. At the same time 62.13% to 63.22% of deltamethrin still persisted in the control soil samples without the strain XQ08. Microorganisms degrades the xenobiotic compounds and produces certain metabolites that is either toxic or non-toxic to the environment. In this study, the formation of metabolites were detected by qualitative analysis through GC-MS. Two compounds, A and B, were detected in the present study.. Compound A with significant peaks at retention time 3.97 with m/z was identified as a metabolite with phenol group, and compound B with significant peaks at retention time 8.22 was identified as a metabolite with ester group butyl-2-ethyl hexyl ester. Some researchers determined that pyrethroid pesticide metabolites formed as a result of degradation by microorganisms. They indicated that esters are considered susceptible to degradation by microbes via hydrolysis which is the main step in the detoxification of Pyrethroids (Hu et al., 2018). Zhao et al., (2016) reported that the degradation of beta-cypermethrin by *Bacillus licheniformis* B-1 resulted in the hydrolysis of beta-cypermethrin to 3-PBA. *Pseudomonas sp.* strains could utilize and transform 3-PBA (Topp and Akhtar, 1991; Halden et al., 1999). Tang et al., (2020) verified the degradation mechanisms of deltamethrin and 3- PBA by co-culture of strains LH-1-1 and BPBA052. They determined the metabolite such as (1R, cis)-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane carboxylic acid, phenol and butyl dactyl ester. The formation of phenol may be due to the oxidation and cleavage of diaryl ether of 3-PBA by the strain BPBA052 (Tang et al., 2012). In concord with the findings of earlier research, this study infers that deltamethrin is efficiently decomposed by the strain DRNB1 in both liquid MSM and soil samples.

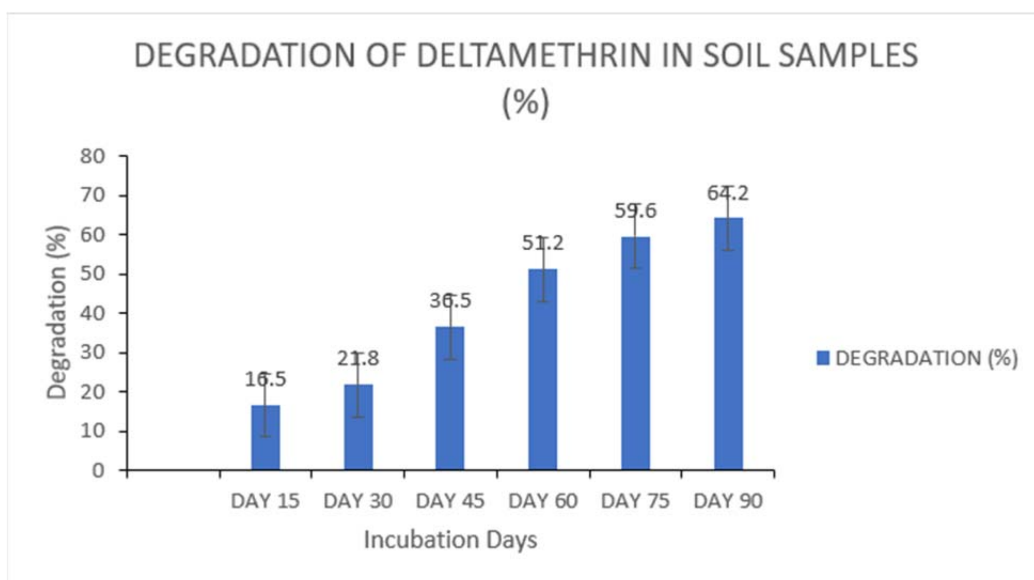


Figure 4.49 Degradation of deltamethrin in soil samples by strain DRNB1. The values are expressed in Mean ± SD. ($p<0.05$).

4.4.2.2 Degradation of ethion in soil samples by *Stenotrophomonas maltophilia* EON2

The study indicated that the degradation of ethion in sterilised soil samples inoculated with EON2 was observed as 12.4%, 18.8%, 46.5%, 56.5%, 68.9%, and 72% during the 15th day, 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, degradation of ethion in control sterilised soil samples without EON2 was extremely low during the 90-day experiment time. Nearly 97.4% of the ethion in the control sterilised samples remains intact, only 2.6% of ethion degradation was observed in the control sample. The degradation frequency of ethion in soil augmented with EON2 indicated that during the first 15 days, the process was slow. Merely 12.4% of the applied dose of ethion was degraded in soils during 15 days. After 15 days the removal of ethion increased considerably giving a final value of 72% in soils inoculated with EON2. The half -life of ethion in soil samples with EON2 was determined as 49.00 days. This could be due to the dependency of ethion degradation on the presence of microorganisms.

Biodegradation studies of ethion are limited. There have only been a few findings on the breakdown of ethion and its metabolites. Dierberg and Pfeuffer, (1983) reported that the half-life of ethion in water is 26 days and this increases with a difference in the pH of the water samples. They also implied that hydrolysis may be a significant mechanism in the loss of ethion in irrigation canal waters in south Florida. Foster et al., (2004) reported that abiotic hydrolytic products of ethion such as ethion monoxon,

ethion dioxon, O,O-diethyl thiophosphate, and thioformaldehyde were not formed in their degradation studies of ethion. They also revealed that ethion differs structurally from parathion and other organophosphate pesticides in that it lacks of an aromatic or heterocyclic group, which makes the degradation of ethion faster. Xia and Ma, (2006) demonstrated the degradation of ethion by water hyacinth in water samples and found that 69% of ethion was removed within one week. All these studies confirm that ethion can be easily and efficiently degraded by microorganisms and plants in both water and soil ecosystems. In the present study, the ethion was degraded only in the presence of strain EON2. This could be attributed to the influence of microbial communities in the degradation process of ethion. The presence of microbial communities as well as microbial activity increases the degradation rate (Yang et al., 2000). Bishnu et al., (2012) analysed the dissipation rate of ethion in tea soils of Kailashpur and reported that ethion has lower half-lives when applied in field rates. The half-life was increased when the application rate of ethion become ten times the field rate. The half-lives were 42.3 to 75.2 from 39 to 70 days in Dooras soils and Hill soils respectively. The percentage of the degradation of ethion was higher at 30°C than at 20°C during the 90 days study period. They also determined that ethion degradation in tea soil was dependent on soil type, temperature, and application rate. Zhu et al., (2002) also reported that lower temperature, and higher concentration of ethion resulted in the higher persistence of ethion in soils. In the present study, no metabolites of ethion were detected in GC-MS analysis. From the above explained reports it is inferred that the degradation of ethion not only depends on the microbial communities but also on environmental factors like temperature, pH, and their application rate.

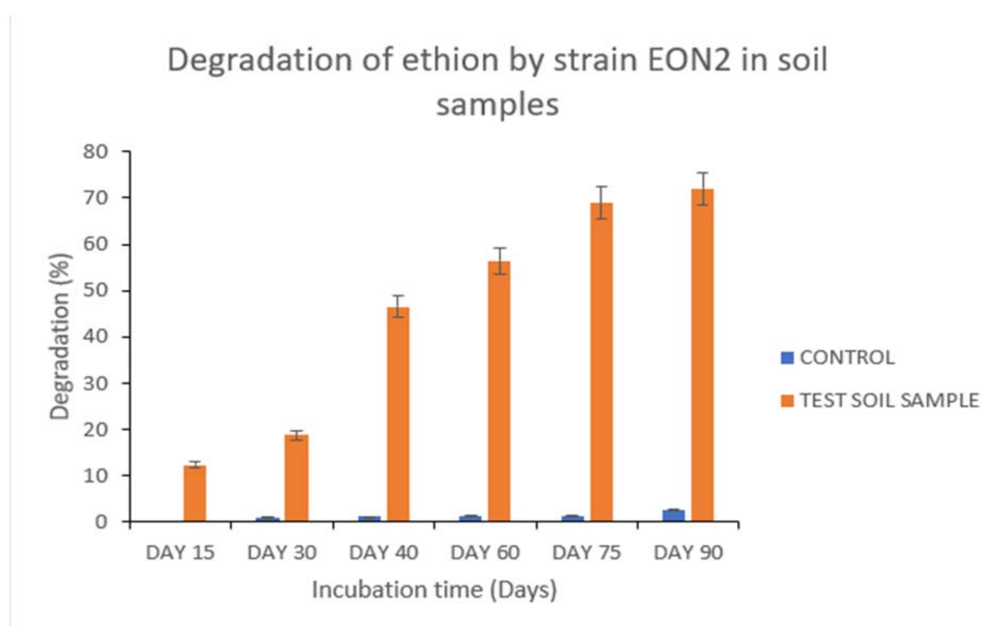


Figure 4.50 Degradation of ethion in soil samples by strain EON2. The values are expressed in Mean ± SD. ($p<0.05$).

4.4.2.3 Degradation of thiamethoxam in soil samples by *Stenotrophomonas maltophilia* TXM2

The study showed that the degradation of thiamethoxam in sterilised soil samples inoculated with TXM2 was observed as 11.2%, 18.6%, 21.9%, 36.5%, 41.9% and 47.8% during 15th , 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, the degradation of thiamethoxam control sterilised soil samples without TXM2 was extremely low during the 90-day experiment time. Nearly 96% of the thiamethoxam in the control sterilised samples remained intact, only 4% of thiamethoxam degradation was observed in the control sample. The degradation frequency of thiamethoxam in soil augmented with TXM2 indicated that during the first 15 days the process was slow. Merely 11.2% of the applied dose of thiamethoxam was degraded in soils during 15 days. After 15 days, the removal of thiamethoxam increased considerably giving a final value of 47.8% in soils inoculated with TXM2. The half-life of thiamethoxam in soil samples with TXM2 was determined as 95.96 days. This could be due to the dependency of thiamethoxam degradation on the presence of microorganisms. The increased rate of biodegradation and decrease in half-life (95.96days) in soil inoculated with strain TXM2 can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples, the removal rate of thiamethoxam was only 4% and the half-life was

1528.1 days which is approximately 15 times more than in soil samples inoculated with TXM2. This could be attributed to the presence of microorganisms in the soil that enhance the process of degradation. This fact was also observed in some previous studies. For example, Rana et al., (2015) reported that B.aeromonas strain IMBL 4.1 and pseudomonas putida strain IMBL 5.2 degraded 88.52 to 96.27% of 0.01 to 0.50 mg/kg of thiamethoxam in clay loam soil. At the same time, in the soil unamended with bacteria strains, the thiamethoxam persisted for more than 56 days. Hegde et al., (2017) isolated three bacterial strains THIA3, THIA4, and THIA 7 that degrade thiamethoxam 75.41%, 68.22%, and 63.23% respectively in soils inoculated with these strains. However, in control samples without bacteria strains the thiamethoxam degradation observed was only 42.94%. Both these studies imply that the presence of microorganisms enhances the degradation of thiamethoxam to its maximum level, which is in agreement with the present study. The thiamethoxam persistence rate depends on the soil type. The persistence of thiamethoxam in the soil is quite clear. It is known to persist in sandy loam soil with a half-life of 16.9 days under laboratory conditions (Karmakar et al., 2009) and 19.2 days in light conditions (Gupta, 2006). Microorganisms degrade the pesticides and result in the formation of different metabolites in many cases.Pandey et al., (2009) studied the degradation of thiamethoxam by *Pseudomonas sp*. 1G and identified the degradation products such as nitrosoamino, Nitrosoguanidine, and urea. Zhou et al., (2013) also identified the degradation metabolites of thiamethoxam by *Ensifer adhaerens* TMX-23 such as nitrosoimino and urea. All these reports on thiamethoxam degradation and findings of the present study indicate that the degradation of thiamethoxam is not primarily dependent upon the presence of microorganisms. The degradation of thiamethoxam is affected by all the environmental factors and the type of soil it is applied. It also denotes that *Stenotrophomonas maltophilia* strain TXM2 can be used for the effective degradation of thiamethoxam in contaminated sites.

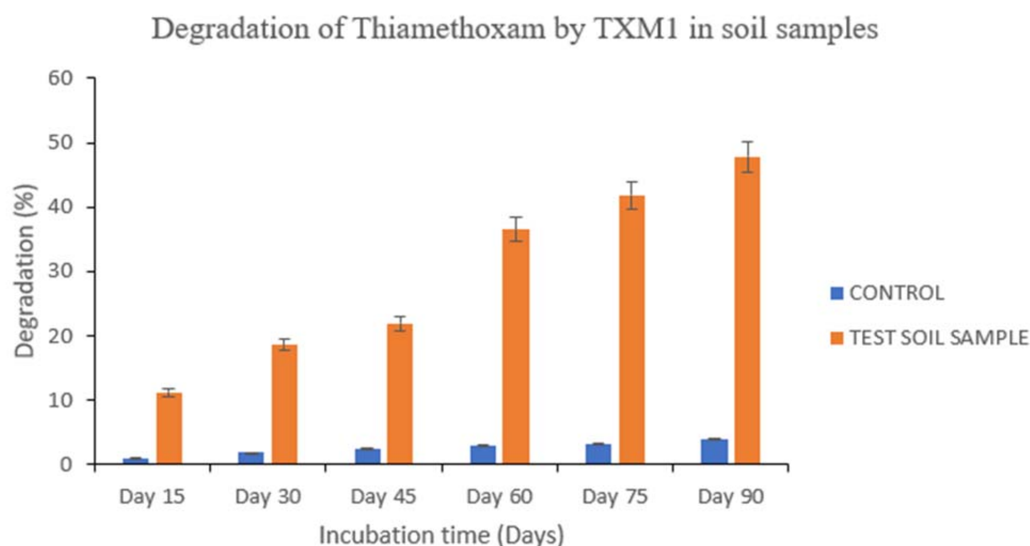


Figure 4.51 The degradation of thiamethoxam in soil samples by the strain TXM1. Values are expressed in Mean ± SD ($p<0.05$).

4.4.2.4 Degradation of spiromesifen by *Stenotrophomonas[pseudomonas] geniculata* SFN1

The present study showed that the degradation of spiromesifen in sterilised soil samples inoculated with SFN1 was observed as 28%, 32.6%, 49.8%, 52.6%, 61.95%, and 68% during the 15th day, 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, the degradation of spiromesifen control sterilised soil samples without SFN1 was extremely low during the 90-day experiment time. Nearly 91.5% of the spiromesifen in the control sterilised samples remained intact, only 8.5% of spiromesifen degradation was observed in the control sample. The degradation frequency of spiromesifen in soil augmented with SFN1 indicated that during the first 15 days, the process was slow. Merely 28% of the applied dose of spiromesifen was degraded in soils during 15 days. After 15 days the removal of spiromesifen increased considerably giving a final value of 68% in soils inoculated with SFN1. The half-life of spiromesifen in soil samples with SFN1 was determined as 54.74 days. This could be due to the dependency of spiromesifen degradation on the presence of microorganisms. The increased rate of biodegradation and decrease in half-life (54.74 days) in soil inoculated with strain SFN1 can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples, the removal rate of spiromesifen was only 8.5% and the half-life is 702.26 days which is approximately 12 times more than in the soil samples inoculated with SFN1. This

could be attributed to the presence of microorganisms in the soil that enhance the process of degradation.

The biodegradation studies of spiromesifen by microorganisms in both liquid media and soil is very limited. Mate *et al*., (2015) reported that the persistence of spiromesifen in soil is influenced by moisture, light, compost amendment, soil sterilization and pH. They also studied the kinetic modelling of spiromesifen degradation in soil and found that it follows first order kinetics and observed that compost amendment in soil enhanced the dissipation of spiromesifen. This may be due to the presence of microorganisms in compost which enhances the degradation of spiromesifen. This is in agreement with the present study where the degradation of spiromesifen in soil amended with SFN1 increases the degradation in comparison with the control sample without strain SFN1. Raj et al., (2021) analysed the soil samples on 20th day after the last spray of spiromesifen and observed that spiromesifen residues are at below quantification limit of 0.01µg/g. Spiromesifen is mostly susceptible to photo-degradation and is more prone to degradation under UV light than sunlight exposure. Enol metabolites are the photodegradation products of spiromesifen. This could be the reason for the absence of enol metabolites of spiromesifen in the present study.

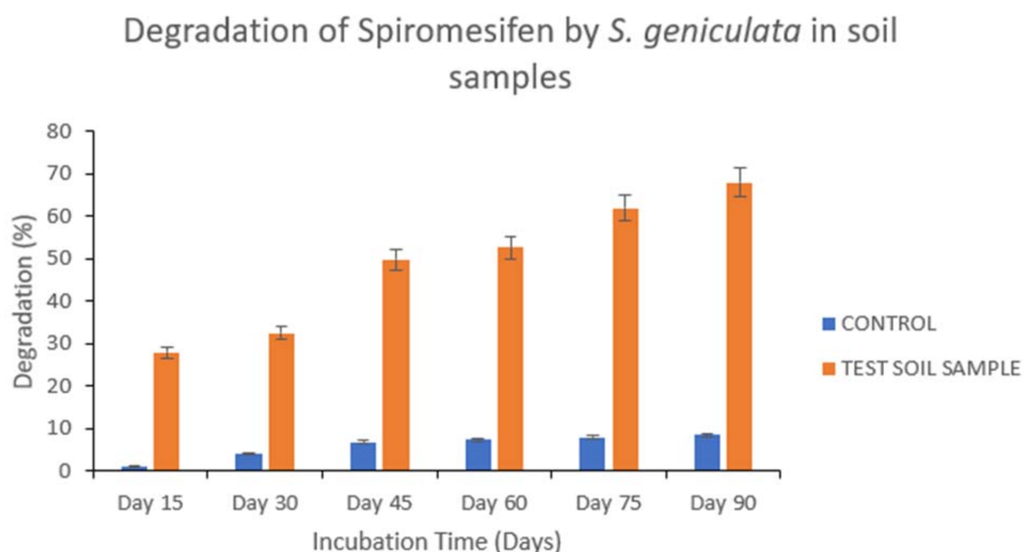


Figure 4.52 The degradation of spiromesifen in soil samples by the strain SFN1. Values are expressed as Mean ± SD ($p<0.05$).

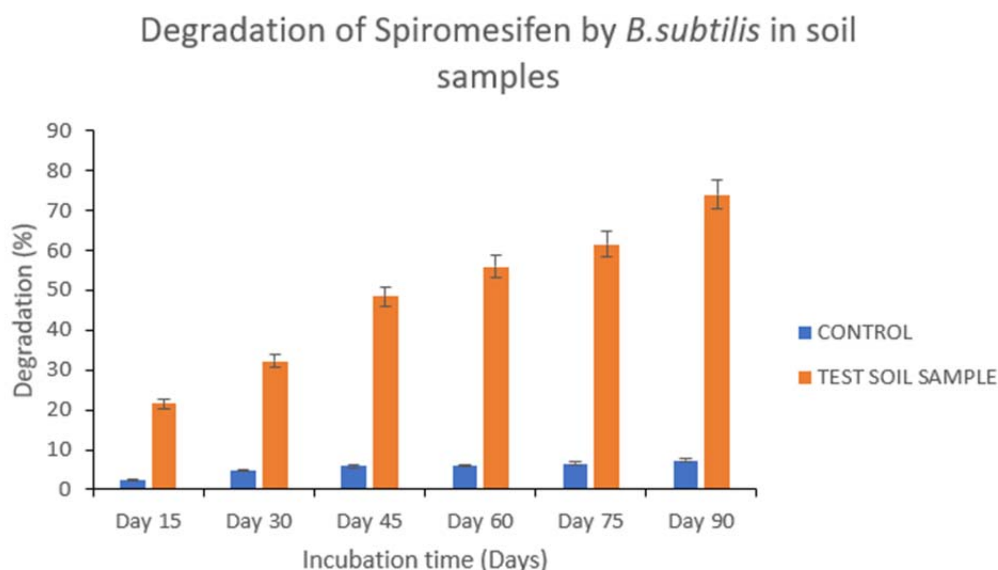


Figure 4.53 The degradation of spiromesifen in soil samples by the strain SFT1. Values are expressed as Mean ± SD ($p<0.05$).

Stenotrophomonas [Pseudomonas] geniculata has much more potential applications in the field of environmental protection. It could utilize and transform xenobiotic compounds like nicotine (Liu et al., 2010), paraquat (Wu et al., 2020). The spiromesifen in the soil amended with strain SFN1 degraded faster than the spiromesifen in the control sample without the strain SFN1. This could be attributable to the ability of the *Stenotrophomonas geniculata* in the degradation process of spiromesifen. Pattanasuttichonlakul et al., (2018) reported that the soil mixture of dairy wastewater sludge added with *P. geniculata* WS3 accelerated the biodegradation of PLA. Boonluksiri et al., (2021) also reported that *P.geniculata* WS3 increases the rate of degradation of PBS and PLA in comparison to the control samples. Both these study reports uphold the observations of this study that *Stenotrophomonas [Pseudomoas] geniculata* has the strongest ability to degrade xenobiotic compounds including the acaricide spiromesifen.

4.4.2.5 Degradation of spiromesifen by *Bacillus subtilis* SFT1

The study showed that the degradation of spiromesifen in sterilised soil samples inoculated with SFT1 was observed as 21.5%, 32.3%, 48.5%, 55.98, 61.6%, and 74% during the 15th day , 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, the degradation of spiromesifen control sterilised soil samples without SFT1 was extremely low during the 90-day experiment time. Nearly 92.7% of the

spiromesifen in the control sterilised samples remained intact, and only 7.3% of spiromesifen degradation was observed in the control sample. The degradation frequency of spiromesifen in soil augmented with SFT1 indicated that during the first 15 days, the process was slow. Merely 21.5% of the applied dose of spiromesifen was degraded in soils during the 15 days. After 15 days, the removal of spiromesifen increased considerably giving a final value of 74% in soils inoculated with SFT1. The half-life of spiromesifen in soil samples with SFT1 was determined as 46.31 days. This could be due to the dependency of spiromesifen degradation on the presence of microorganisms. The increased rate of biodegradation and decrease in half-life (46.31 days) in soil inoculated with strain SFT1 can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples the removal rate of spiromesifen was only 7.3% and the half-life is 822.98 days which is approximately 17 times more than in soil samples inoculated with SFT1. This could be attributed to the presence of microorganisms in the soil that enhances the process of degradation.

The degradation studies of spiromesifen in soil by microorganisms are very limited. Siddamallaih et al., (2017) reported that the rate of dissipation of spiromesifen is higher in soil samples than in leaves and vegetables. This is due to the presence of microorganisms in the soil. They also revealed that no spiromesifen metabolite – spiromesifen -enol was formed during the degradation process. In the present study also, no metabolites were found in the qualitative GC-MS analysis of the soil samples. This could be because all the metabolites were used up by the microbes. It is well known that *Bacillus* is a highly active bacteria and can degrade a wide range of xenobiotic compounds. Several studies recorded the degradation ability of Bacillus sp. strains towards xenobiotic compounds like nicosulfuron (Kang et al., 2010), endosulfan (Kumar et al., 2014), carbendazim (Salunkhe et al., 2014). In the present study, the degradation of spiromesifen was enhanced by the presence of *Bacillus subtilis* strain SFT1. Several studies reported the degradation of pesticides in soil by *Bacillus sp*. Bhatt et al., (2019) reported that *Bacillus thuringensis* SG4 degraded 83.3% of cypermethrin in soil samples after 15 days and decreased the half-life from 177.7 days to 0.7 days. Bhatt et al., (2019) reported that Bacillus sp. degraded 77% of fipronil in soil after 15 days of incubation. Omeiri et al., (2022) found that *Bacillus paramycoides* and *Bacillus proteolyticus* degraded 88.3% to 93.2% of pesticides in the soil samples. Analogous to the previous reports, this study proves beyond doubt

that *Bacillus sp*. is one of the prime bacteria for the degradation of xenobiotic compounds.

4.4.2.6 Degradation of fenpyroximate by *Paenibacillus alvei* F1T

The study showed that the degradation of fenpyroximate in sterilised soil samples inoculated with F1T was observed as 11.9%, 28.7%, 32.65%, 41.26%, 58.6%, and 62.2% during 15th , 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, the degradation of fenpyroximate control sterilised soil samples without F1T was extremely low during the 90-day experiment time. Nearly 97.7% of the fenpyroximate in the control sterilised samples remained intact, and only 2.3% of fenpyroximate degradation was observed in the control sample. The degradation frequency of fenpyroximate in soil augmented with F1T indicated that during the first 30 days, the process was slow. Merely 28.7% of the applied dose of fenpyroximate was degraded in soils during 30 days. After 30 days the removal of fenpyroximate increased considerably giving a final value of 62.2% in soils inoculated with F1T. The half-life of fenpyroximate in soil samples with F1T was determined as 64.12 days. The increased rate of biodegradation and decrease in half-life (64.12 days) in soil inoculated with strain F1T can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples, the removal rate of fenpyroximate was only 2.3% and the half-life is 2681 days which is approximately 41 times more than in soil samples inoculated with F1T. This could be attributed to the presence of microorganisms in the soil that enhance the process of degradation.

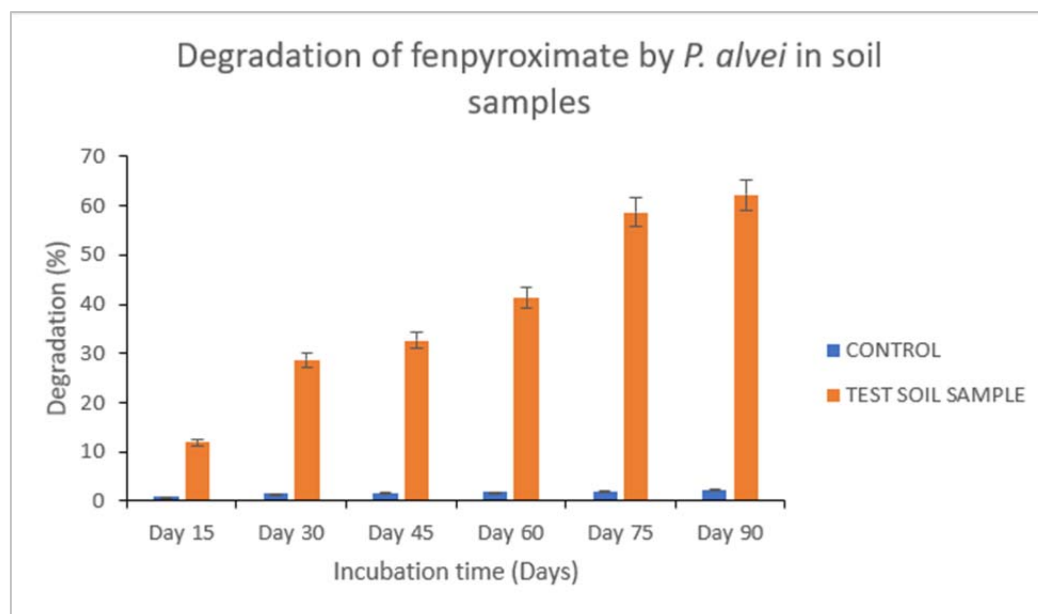


Figure 4.54 degradation of fenpyroximate in soil samples by the strain F1T. Values are expressed as Mean ± SD ($p<0.05$)

The degradation studies of fenpyroximate by microorganisms and in soil are very rare. The only report available was by Li ZengMei et al., (2009) who studied the degradation dynamics of fenpyroximate in cotton fields. They reported that the half-life of fenpyroximate in the soil of open cotton fields was 8.8 to 9.6 days due to the action of indigenous microorganisms present in the soil samples. They also observed that the residue level of fenpyroximate was in the range of 0.01 to 0.98 mg/kg in soil. this is in agreement with the present study where the half- life of fenpyroximate in soil samples with F1T is 41 times lower than the control samples without the strain F1T. The xenobiotic biodegradation capacity of *Paenibacillus sp*. in soil was reported earlier by several researchers. For example, Daane et al., (2001) reported that a consortium consisting of *Paenibacillus sp*. strain enhanced the degradation of polycyclic aromatic hydrocarbons in marine sediment slurry in comparison with control samples. Birolli et al., (2020) also reported that the consortia consisting of *Paenibacillus sp*. improved the degradation of fungicide pyraclostrobin in soil samples. This consortium reduced the 100 mg/L of fungicide to 57.2 mg/L at 30°C, within 28 days. The degradation rate was slow during the initial days and then increased considerably, which is due to the period required for the bacteria to attain the exponential phase. This could be the reason for the degradation of fenpyroximate by strain F1T which was also low during the first 30 days, and then exhibited an increase in the degradation levels in the present study. The findings of all these studies

along with the present study imply that *Paenibacillus alvei* strain F1T is one of the efficient bacteria for the biodegradation of xenobiotic compounds.

4.4.2.7 Degradation of fenpyroximate by *Pseudomonas aeruginosa* FXE1

In this study, the degradation of fenpyroximate in sterilised soil samples inoculated with FXE1 was observed as 8.9%, 16.81%, 27.56%, 39.72%, 51.25%, and 60% during 15th , 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, the degradation of fenpyroximate control sterilised soil samples without FXE1 was extremely low during the 90-day experiment time. Nearly 97.7% of the fenpyroximate in the control sterilised samples remains intact, only 2.3% of fenpyroximate degradation was observed in the control sample. The degradation frequency of fenpyroximate in soil augmented with FXE1 indicated that during the first 45 days, the process was slow. Merely 27.56% of the applied dose of fenpyroximate was degraded in soils during 45 days. After 45 days the removal of fenpyroximate increased considerably giving a final value of 60% in soils inoculated with FXE1. The half-life of fenpyroximate in soil samples with FXE1 was determined as 68.08 days. The increased rate of biodegradation and decrease in half-life (68.08 days) in soil inoculated with strain FXE1 can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples, the removal rate of fenpyroximate was only 2.3% and the half-life was 2681 days, which is approximately 39 times more than in soil samples inoculated with FXE1 This could be attributed to the presence of microorganisms in the soil that enhance the process of degradation.

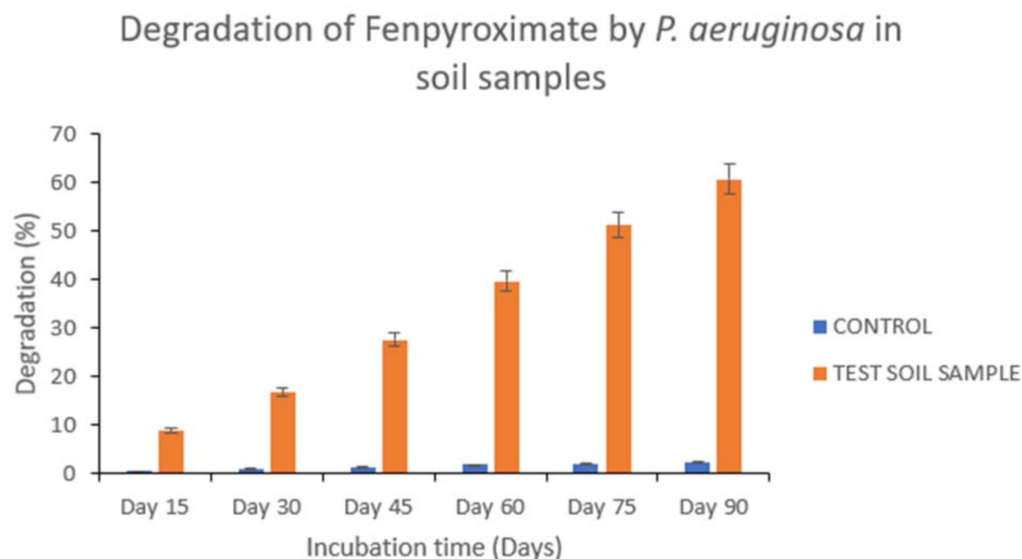


Figure 4.55 degradation of fenpyroximate in soil samples by strain FXE1. Values are expressed as Mean ± SD. ($p<0.05$)

Successful removal of pesticides by inoculation of bacteria *Pseudomonas sp*. in soil samples had been previously reported by researchers. Satapute and Kaliwal, (2016) noticed that *Pseudomonas sp*. degraded 21.95% to 49.85% of propiconazole in soil samples at 27°C. They also observed that the activity of *Pseudomonas sp.* decreased the half-life of propiconazole to 40 days, whereas in control samples, the pesticide degraded only to 39.65%. Both these studies strengthen the findings of the present study and the fact that *Pseudomonas sp.* is an efficient genus for the degradation of various xenobiotic compounds.

4.4.2.8 Degradation of glyphosate by *Acinetobacter baumannii* GLYB2

The present study showed that the degradation of glyphosate in sterilised soil samples inoculated with GLYB2 was observed as 8.2%, 32.1%, 56.5%, 61.2%, 73.8%, and 77.2% during the 15th , 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, degradation of glyphosate in control sterilised soil samples without GLYB2 was extremely low during the 90-day experiment time. Nearly 92.4% of the glyphosate in the control sterilised samples remains intact, and only 7.6% of glyphosate degradation was observed in the control sample. The degradation frequency of glyphosate in soil augmented with GLYB2 indicated that during the first 15 days, the process was slow. Merely 8.2% of the applied dose of glyphosate was degraded in soils during 15 days. After 15 days the removal of glyphosate increased

considerably giving a final value of 77.2% in soils inoculated with GLYB2. The half-life of glyphosate in soil samples with GLYB2 was determined as 42.19 days. The increased rate of biodegradation and decrease in half-life (42.19 days) in soil inoculated with strain GLYB2 can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples, the removal rate of glyphosate was only 7.6% and the half-life is 789.23 days which is approximately 18 times more than in soil samples inoculated with GLYB2. This could be attributed to the presence of microorganisms in the soil that enhances the process of degradation.

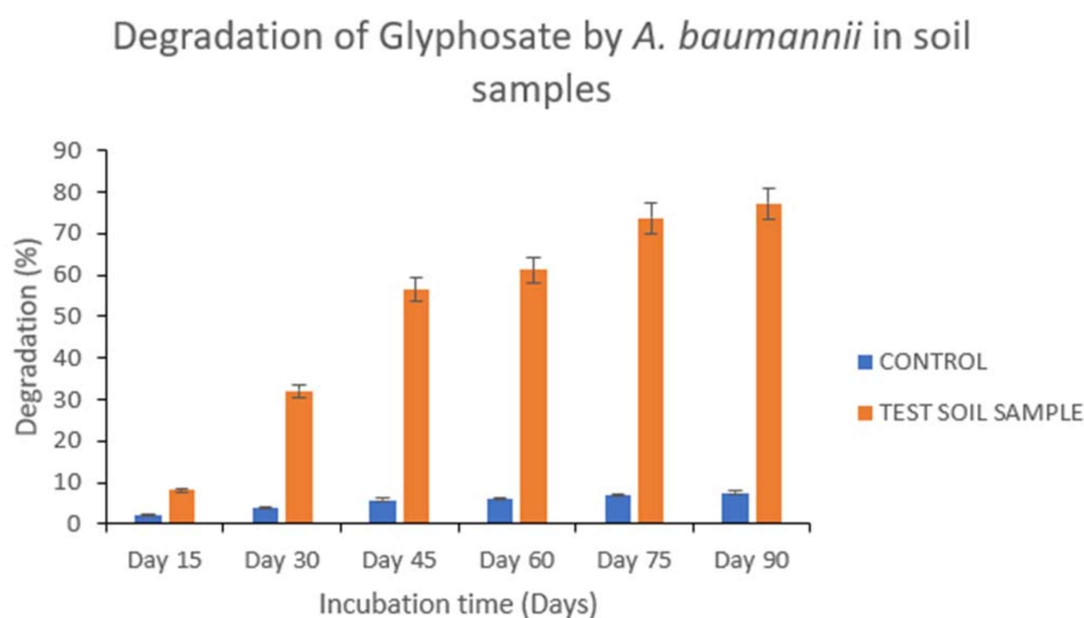


Figure 4.56 Degradation of glyphosate by the strain GLYB2. Values are expressed as Mean ± SD

Several researchers explored the glyphosate degradation ability of different microorganisms in soil samples. For example, Ermakova et al., (2010) reported the glyphosate degradation ability of *Achromobacter s*p. Kg 16, Ermakova et al., (2010) reported that *Achromobacter* sp. Kg16 degraded 65.8% of glyphosate within 21 days. They also stated that *O. anthropic* GPK3 degraded 49.5% of glyphosate within 21 days, and in the control sample without these bacterial inoculums the glyphosate removal rate was only 23.3% within 21 days. This is in agreement with the present study where in the control sample only 7.6% of glyphosate was removed after 90 days. The degradation of glyphosate depends on microbial decomposition, adsorption process, and mineralization (Sviridov et al., 2014). Zhao et al., (2015) also reported

the degradation of glyphosate by *Pseudomonas sp*. in soil samples with an incubation period of 18 days. The range of glyphosate degradation was in the range of 53.6% to 80.8% in samples inoculated with bacteria, and 27.2% in control samples without the inoculum. These strains also improved the quality of contaminated soil by reducing the half-life in the range of 7.43 to 16.07 days in the soil samples inoculated, in comparison with the control samples. In their study, a high rate of glyphosate degradation was observed in the first six days. However, in the present study, the degradation rate was very low during the first 15 days. This could be attributed to the time required for the strain GLYB2 to adapt to the growing media. Sun et al., (2019) observed that more than 85% of glyphosate was degraded within 35 days in the presence of microorganisms in soil samples and the glyphosate residue persisted in the soil until 175 days. They also found that the half-life of glyphosate varied in the range of 28.9 to 31.5 days in the inoculated soil samples, whereas no degradation was observed in the sterilized control soil sample. Li et al., (2022) noticed that *Stenotrophomonas acidaminiphila* Y_4B degraded 71.93 to 89.81% glyphosate within five days. All these study reports substantiate the present study that the degradation of glyphosate in soil is entirely dependent on the activity of microorganisms. The microorganisms in the soil metabolise the glyphosate in the soil and reduce its half-life for rapid dissipation. Another factor that affects the degradation of glyphosate in soil is the texture of the soil. Sorensen et al., (2006) reported that glyphosate degradation in clay soils varied from 9.3% to 14.7% for a period of three months. On the contrary, glyphosate degradation in sandy soils only 2% of removal occurred within a month (Strange-Hansen et al.,2004). However, in the present study, in sandy soils, the degradation of 77.2% was attained after three months. This could be attributed to the difference in bacterial species isolated and the environmental factors of different ecosystems. The glyphosate is degraded via two pathways, like aminomethylphosphonic acid pathway and sarcosine/glycine pathway. In the present study, a peak was observed in the GC-MS qualitative analysis of the soil sample inoculated with GLYB2 at the retention time 19.59, and it is identified as glycine. Similar degradation pathways and sarcosine/glycine formation by the activity of microrganisms was observed by other researchers also. For example, Pipke et al., (1987) reported that Arthrobacter sp. GLP1 degraded glyphosate to glycine; Liu et al., (1991) observed the formation of sarcosine and glycine after the degradation of glycine. Zhang et al., (2022) reported that the bacterial spp. Metabolises glyphosate

by breaking the C-P bond and results in the formation of sarcosine and glycine. Sun et al.,(2019) also reported the formation of glycine as the glyphosate degradation product. All these studies validate the findings of the present study that glyphosate was metabolised by the strain GLYB2 by producing glycine as the intermediate.

Previous reports state that *Acinetobacter sp*. has a high potential for the degradation of pesticides in soil samples. Hussaini et al., (2013) reported that *Acinetobacter sp*. degraded 38% of chlorpyrifos in soil samples within a short period of time. Zhao et al., (2014) also reported that *Acinetobacter sp.* degraded 60.2% of chlorpyrifos in sterilised soils after 18 days of incubation. Zhan et al., (2018) reported that Acinetobacter sp. ZH-14 degraded 85.1% of permethrin in sterilised soil samples by reducing its half-life to 4.9 days within 9 days. In control samples without the bacteria, the degradation was only 21.4% within 9 days. It also degraded butachlor and propanil in soil samples in a short period of time. Tang et al., (2020) reported that *Acinetobacter sp*. degraded 77.3% of 20mg/kg deltamethrin in 7 days in soil samples. All these study reports and findings of the present study reveal that *Acinetobacter sp.* is a potential and efficient bacterium for the bioremediation of glyphosate in contaminated sites.

4.4.2.9 Degradation of propargite by *Chryseobacteirum cucumeris* PTEB2

The present study showed that the degradation of propargite in sterilised soil samples inoculated with PTEB2 was observed as 49.6%, 52.1%, 59.6%,68.1%, 72.1%, and 85.4% during the 15th , 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, degradation of propargite in control sterilised soil samples without PTEB2 was extremely low during the 90-day experiment time. Nearly 88.6% of the propargite in the control sterilised samples remains intact, and only 11.4% of propargite degradation was observed in the control sample. The degradation frequency of propargite in soil augmented with PTEB2 indicated a high rate of degradation during the first 15 days. Approximately 49.6% of the applied dose of propargite was degraded in soils during the 15 days. After 15 days the removal of propargite increased considerably giving a final value of 85.4% in soils inoculated with PTEB2. The half-life of propargite in soil samples with PTEB2 was determined as 32.4 days. The increased rate of biodegradation and decrease in half-life (32.4 days) in soil inoculated with the strain PTEB2 can be explained by the fact that this

strain increases the catabolic potential of the soil. In control samples, the removal rate of propargite was only 11.4% and the half-life was 515.4 days which is approximately 15 times more than in soil samples inoculated with PTEB2. This could be attributed to the presence of microorganisms in the soil which enhances the process of degradation.

The degradation of propargite in soil by microorganisms is very limited. Researchers analysed the degradation of propargite in fruits, plants, and in open fields. Kumar et al., (2005) observed that the half-life of propargite in tea and apples ranged between 1.66 to 2.61 days. They also reported that the half-life of propargite in soil ranged from 43 to 45 days. Varghese et al., (2011) found that propargite residues persisted in chilli fruits for 5 days and their half-life was 0.63 days after the pesticide application. Sreenivasan et al., (2015) reported that the half-life of propargite in tea leaves varies from 1.63 to 1.92 days. The only available study on the degradation of propargite by bacteria is of Soumik et al., (2010), where they isolated *Pseudomonas sp*. From tea rhizosphere from the same ecosystem of the present study, that degrades 71.9% of propargite in liquid MSM. However, in the present study the isolated bacteria sp. was *Chryseobacterium cucumeris* PTEB2 that degrades propargite 85.4% in soil samples. The present study results revealed that bioaugmentation of propargote contaminated soils with strain PTEB2 enhanced the removal rate of propargite and decreased its half-life in comparison with the control samples.

4.4.2.10 Degradation of quinalphos by *Paenibacillus alvei* Q1T

The present study showed that the degradation of quinalphos in sterilised soil samples inoculated with Q1T was observed as 16.5, 27.8%, 35.2%, 41.9%, 52.8%, and 64.2% during the 15th, 30th day, 45th day, 60th day, 75th day and 90th day respectively. In contrast, degradation of quinalphos in control sterilised soil samples without Q1T was extremely low during the 90-day experiment time. Nearly 96.8% of the quinalphos in the control sterilised samples remained intact, and only 3.2% of quinalphos degradation was observed in the control sample. The degradation frequency of quinalphos in soil augmented with Q1T indicated a slow rate of degradation during the first 30 days. Merely 27.8% of the applied dose of quinalphos was degraded in soils during 30 days. After 30 days, the removal of quinalphos increased considerably giving a final value of 64.2% in soils inoculated with Q1T. The half-life of quinalphos in soil samples with Q1T was determined as 60.73 days. The increased rate of biodegradation and decrease in half-life (60.73 days) in soil inoculated with strain

Q1T can be explained by the fact that this strain increases the catabolic potential of the soil. In control samples, the removal rate of quinalphos was only 3.2% and the half-life is 1918.1 days which is approximately 31 times more than in soil samples inoculated with Q1T. This could be attributed to the presence of microorganisms in the soil that enhances the process of degradation.

Researchers studied the degradation pattern of quinalphos and its metabolites formation by bacteria in liquid MSM. Bhadbhade et al., (2002) reported that both the bacteria sp. Arthrobacyer atrocyaneus MCM B-425 and Bacillus megaterium MCM B-423 degraded quinalphos. They also identified the degradation metabolites as valeric or acetic acid and methylamine. Dhanjal et al., (2014) reported that *Bacillus sp*. and *Pseudomonas sp.* degraded quinalphos in liquid MSM and formed the metabolite dihydroxy quinalphos oxon. Talwar et al., (2014) isolated *Ochrobactrum sp.* strain HZM degrades quinalphos and results in the formation of metabolites 2-hydroxyquinoxaline and diethyl phosphate. Nair et al., (2015) demonstrated the biosurfactant mediated degradation of quinalphos by *Pseudomonas aeruginosa* and resulted in the formation intermediate phosphorothioic acid. In the present study also, the metabolites were analysed by the qualitative analysis of GC-MS and observed a peak at the retention time 6.09 and identified as phosphorothioic acid. The formation of phosphorothioic acid may be due to the hydrolysis of P-O alkyl or aryl bonds. Degradation or detoxification of organophosphorus pesticides by the action of microorganisms is generally through the hydrolysis of P–O alkyl and P–O aryl bonds. Such degradation makes the compound more vulnerable to further metabolism (Ortiz-Hernadez and Sanchez-Salinas, 2011). The data on the degradation of quinalphos in soil by microorganisms and the half-life of quinalphos is very limited. Pandey and Singh, (2004) treated the soil samples with quinalphos for 45 days and reported the half-life ranged from 13.2 to 20.6 days. In the present study, the half-life of quinalphos ranged from 60.73 to 1918.1 days in treated and control samples. From these studies, it is evident that the half-life of quinalphos is dependent on the activity of microorganisms in the soil.

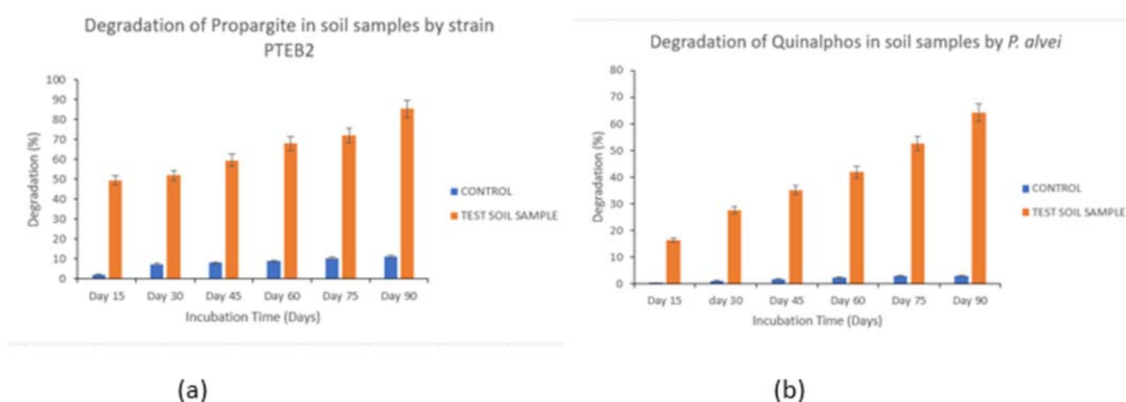


Figure 4.57 Degradation of (a) Propargite by strain PTEB2 & (b) Degradation of quinalphos by strain Q1T

Previously, several researchers reported on *Paenibacillus* sp.'s ability to biodegrade xe nobiotics in soil. For example, Daane et al., (2001) reported that a consortium consisting of *Paenibacillus* sp. strain enhanced the degradation of polycyclic aromatic hydrocarbons in marine sediment slurry in comparison with control samples. Birolli et al., (2020) also reported that the consortia consisting of *Paenibacillus* sp. improved the degradation of fungicide pyraclostrobin in soil samples. This consortium reduced the 100 mg/L of fungicide to 57.2 mg/L at 30°C, within 28 days. The degradation rate was slow during the initial days, and then it increased considerably, which is due to the period required for the bacteria to attain the exponential phase. This could be the reason for the low degradation of quinalphos by strain Q1T during the first 30 days, following which it exhibited an increase in the degradation levels as observed in the present study. All the above findings imply that *Paenibacillus alvei* strain Q1T is one of the efficient bacteria for the biodegradation of xenobiotic compounds.

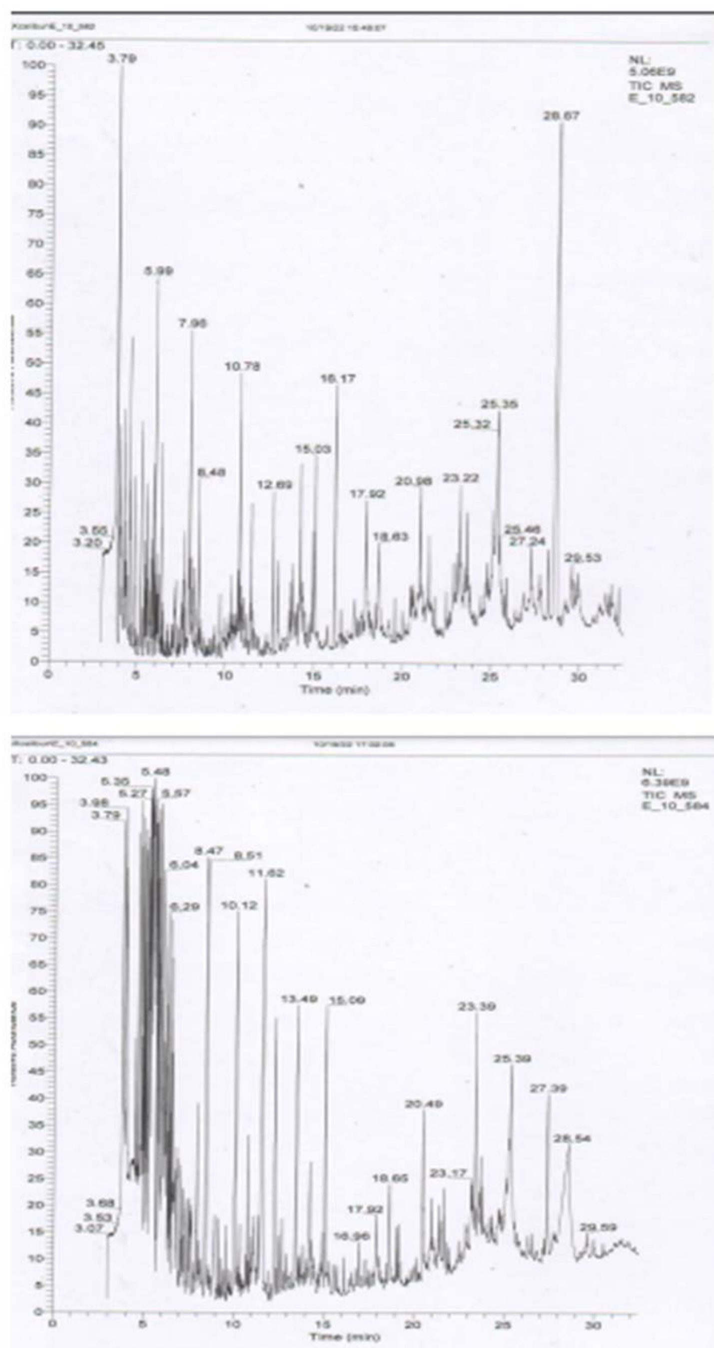


Figure 4.58 GCMS chromatogram of control samples of Deltamethrin & Fenpyroximate, without respective bacterial strains showing the peaks, and retention time.

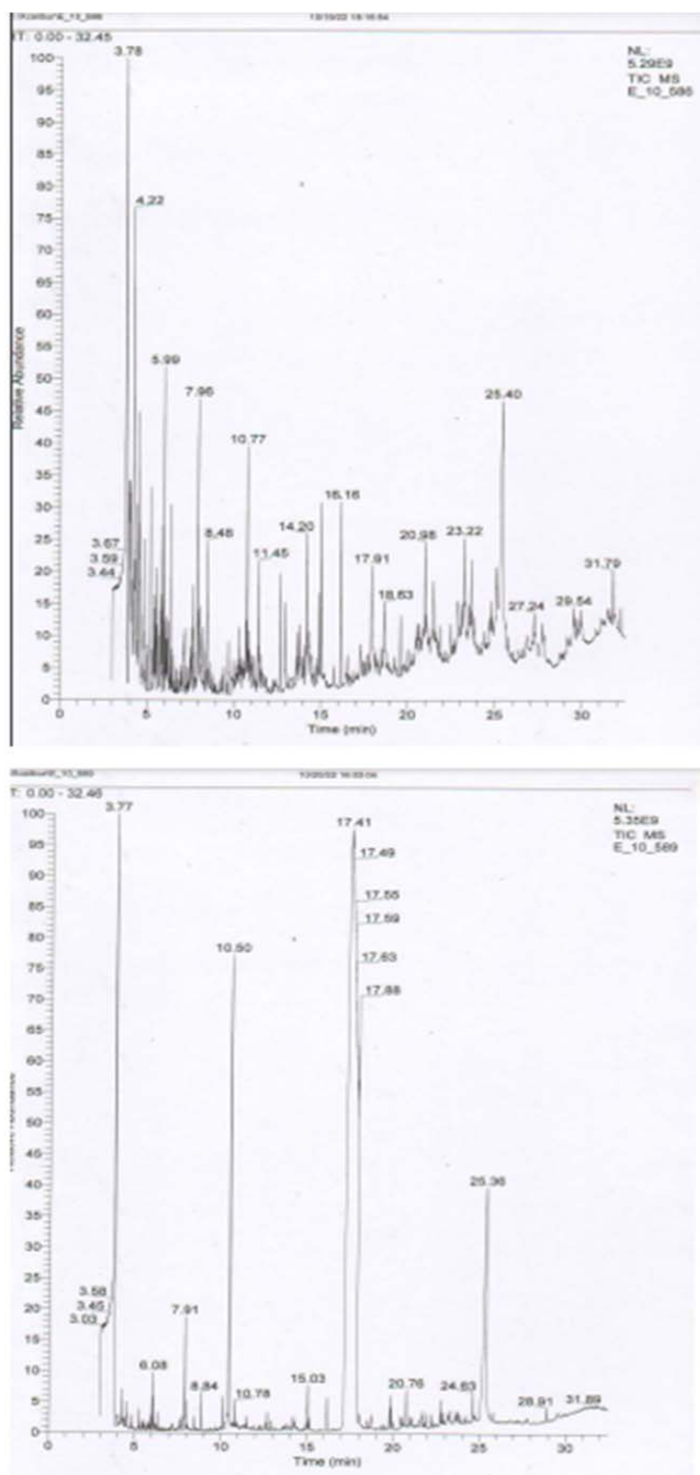


Figure 4.59 GCMS chromatogram of control samples of Glyphosate & Quinalphos, without the respective bacterial strains showing the peaks, and retention time.

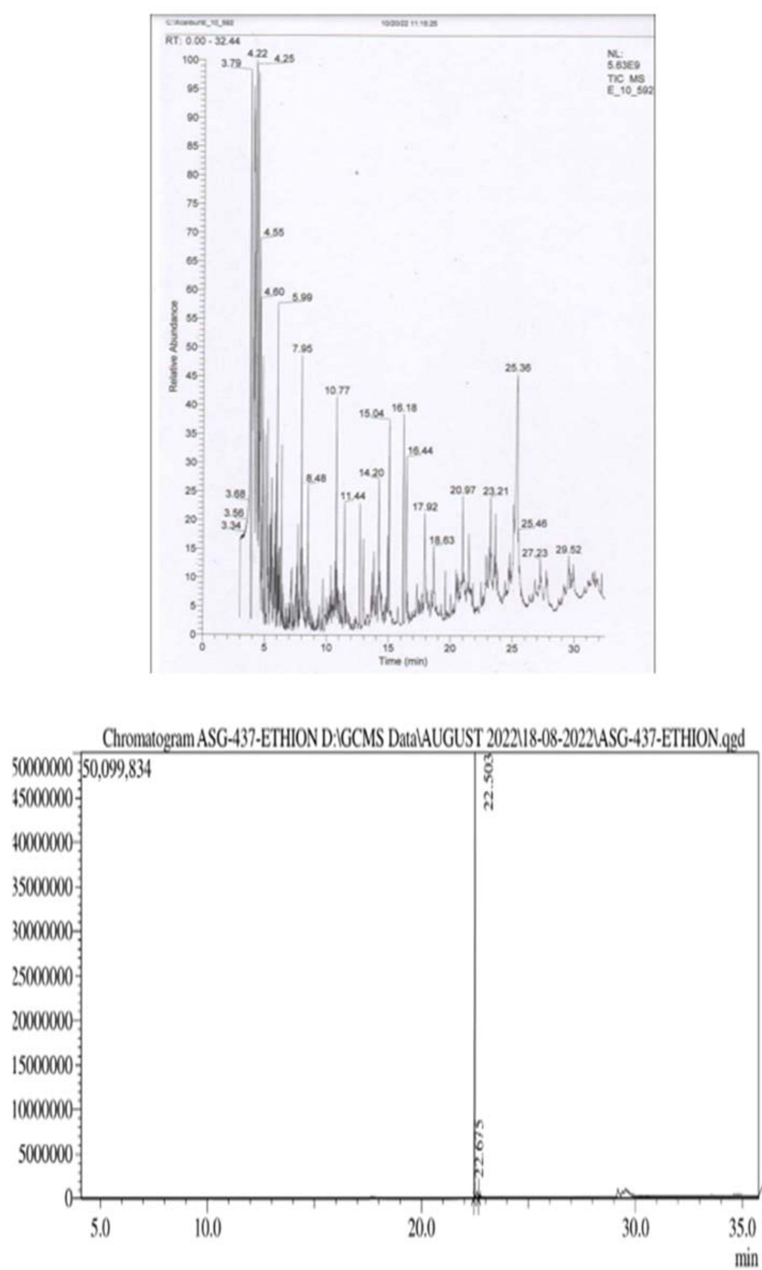


Figure 4.60 GCMS chromatogram of control samples of Thiamethoxam & Ethion,with out the respective bacterial strains showing the peaks, and retention time.

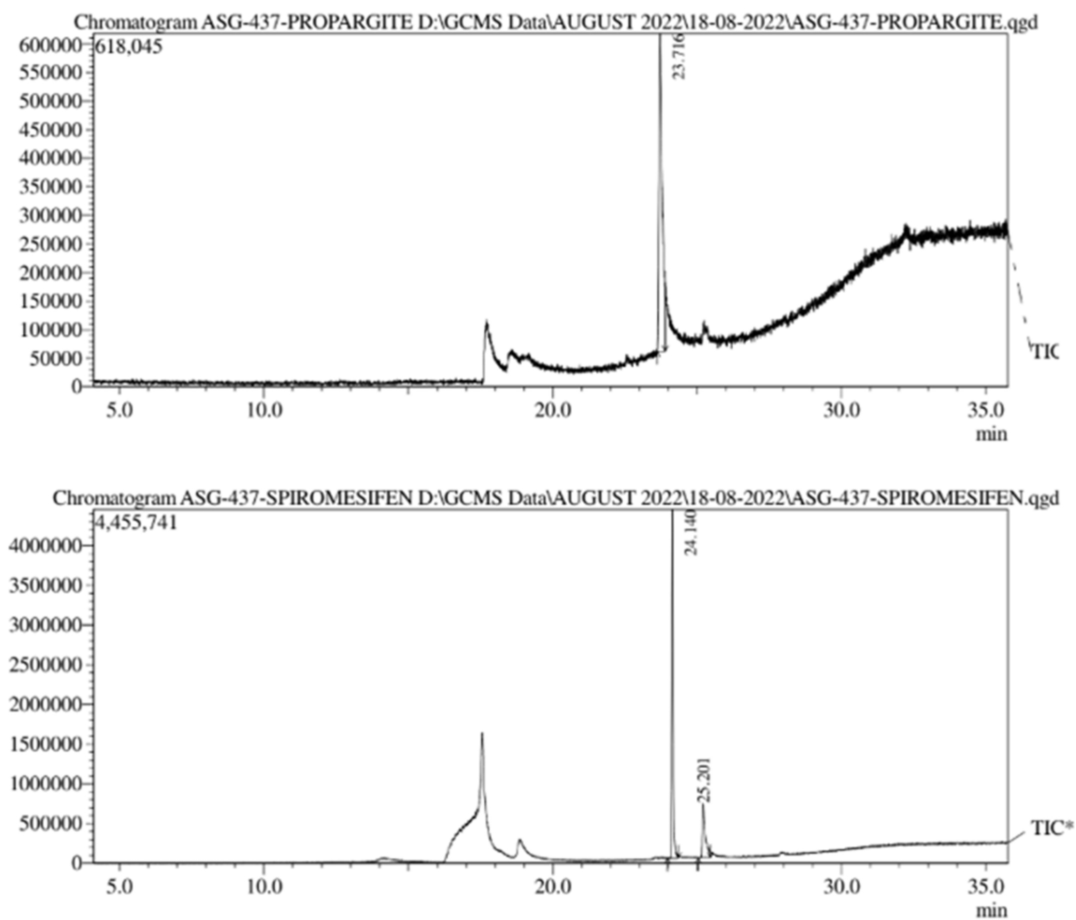


Figure 4.61 GCMS chromatogram of control samples of propargite & Spiromesifen, without the respective bacterial strains showing the peaks, and retention time.

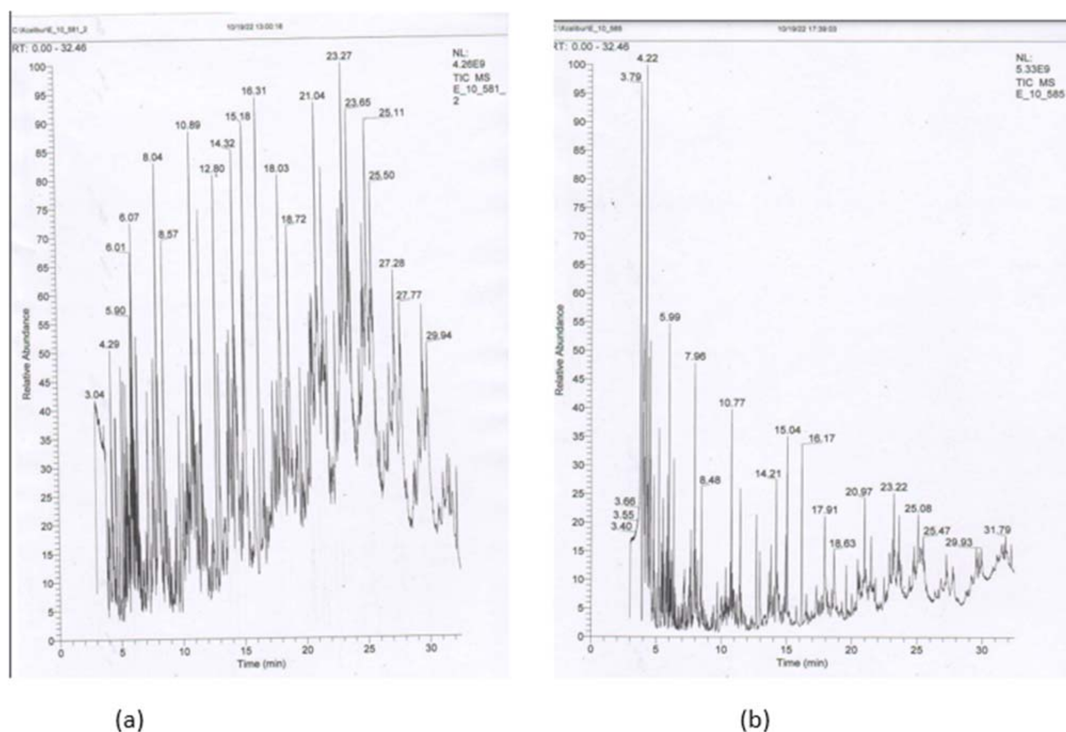


Figure 4.62 GCMS chromatogram of test samples of (a) deltamethrin by bacterial strain DRNB1 & (b) Fenpyroximate by bacterial strain FXE1 , showing the peaks, and retention time.

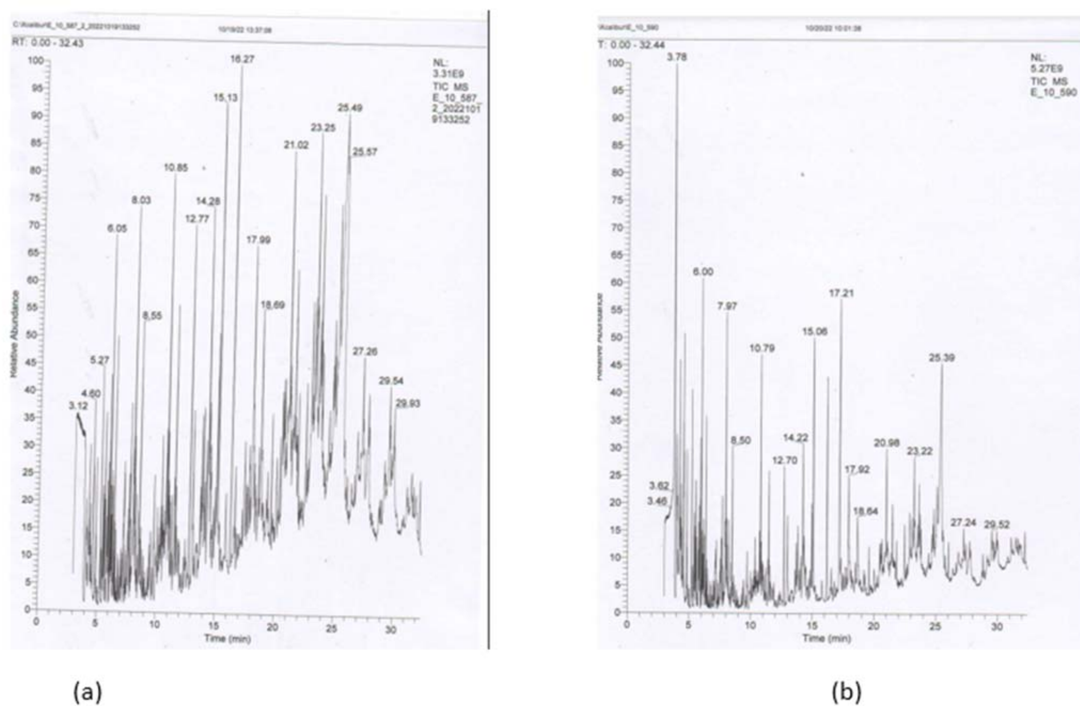


Figure 4.63 GCMS chromatogram of test samples of (a) glyphosate by bacterial strain GLYB2 & (b) quinalphos bacterial strain Q1T, showing the peaks, and retention time.

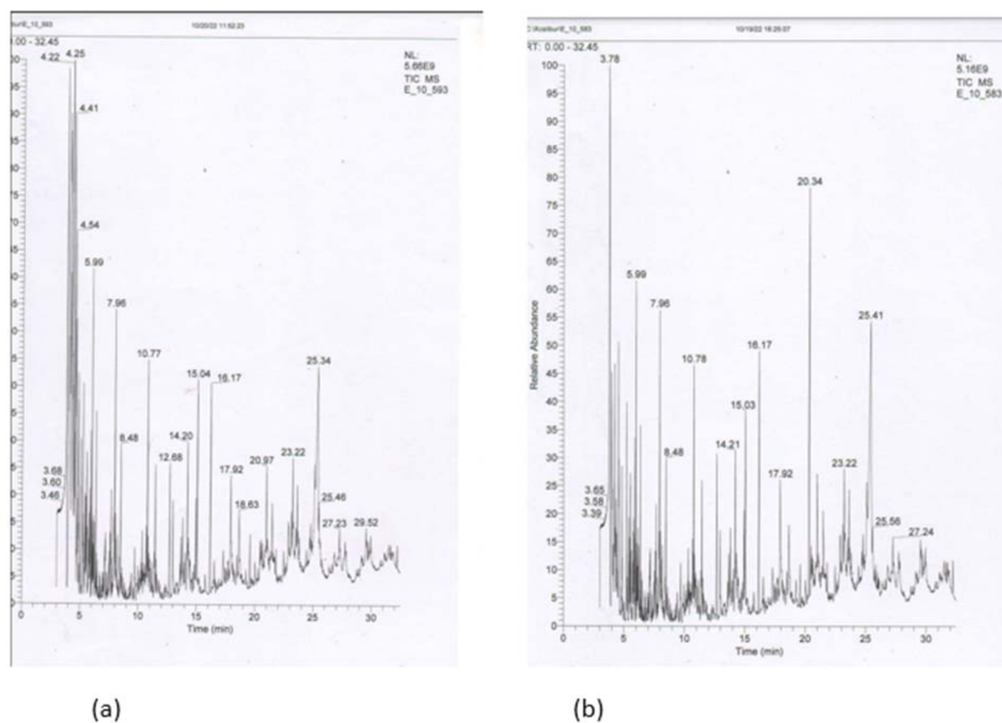


Figure 4.64 GCMS chromatogram of test samples of (a) thiamethoxam by bacterial strain TXM2 & (b) ethion by bacterial strain EON2, showing the peaks, and retention time.

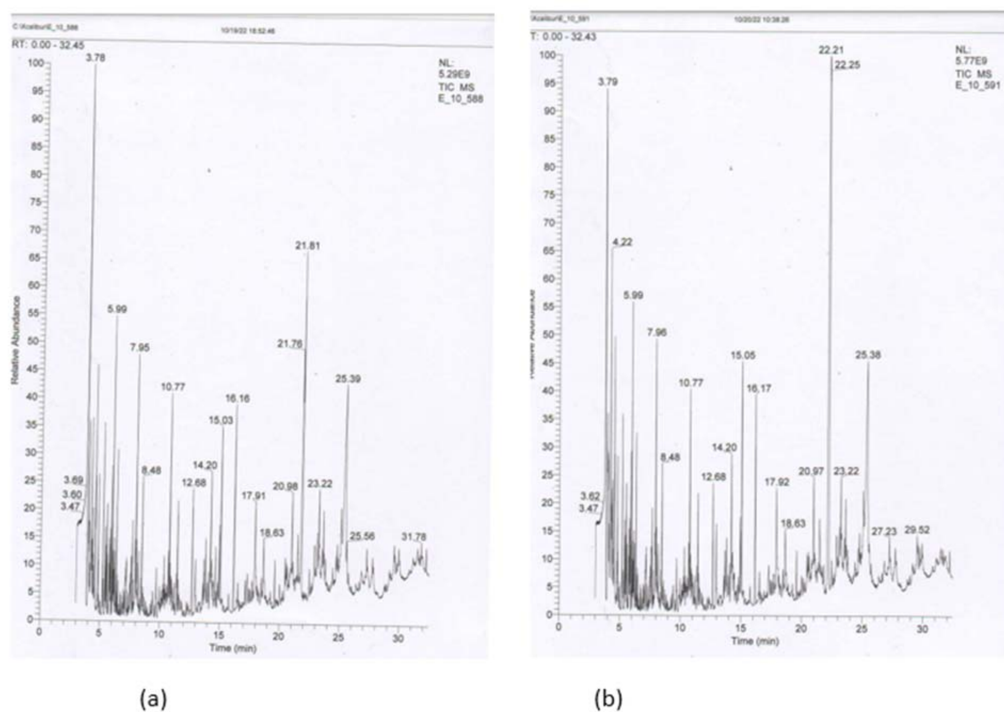


Figure 4.65 GCMS chromatogram of test samples of (a) propargite by bacterial strain PTEB2 & (b) Spiromesifen by bacterial strain SFN1, showing the peaks, and retention time.

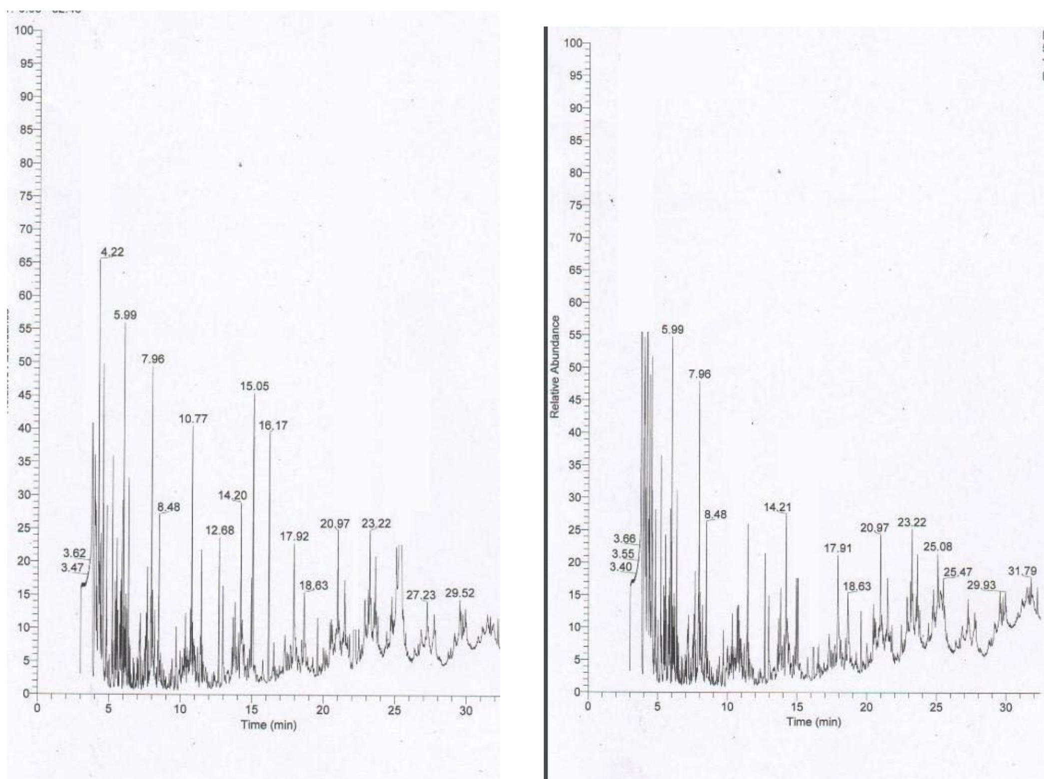


Figure No 4.71: GCMS chromatogram showing peaks of degradtion of fenpyroximate by strain F1T and degradation of spiromesifen by strain SFT1

Table 4.36 The L9 (3)4 Orthogonal experimental results of degradation of fenpyroximate by *P.aeruginosa*

Runs	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$ (days)	Regression equation	R^2
1	25	6	1.5	74.456	37.4341	5.07	$Ln(C_t/C_o) = -18.382x + 2.51$	0.98
2	25	7	2.0	78.1879	37.8700	4.55	$Ln(C_t/C_o) = 55.082x + 5.554$	0.90
3	25	8	2.5	73.0076	37.2640	5.29	$Ln(C_t/C_o) = -128.5x + 32.8$	0.96
4	30	6	2.0	76.026	37.6160	4.85	$Ln(C_t/C_o) = -8.2867x + 11.512$	0.97
5	30	7	2.5	77.8276	37.8192	4.60	$Ln(C_t/C_o) = -8.3755x + 11.596$	0.96
6	30	8	1.5	78.3779	37.8911	4.48	$Ln(C_t/C_o) = -8.307x + 12.098$	0.92
7	35	6	2.5	69.6479	36.8651	5.81	$Ln(C_t/C_o) = -9.0998x + 12.796$	0.92
8	35	7	1.5	77.1776	37.3762	4.69	$Ln(C_t/C_o) = -8.6731x + 11.885$	0.95
9	35	8	2.0	73.9316	37.3729	5.15	$Ln(C_t/C_o) = -8.8594x + 12.073$	0.94

Table 4.37 The L_9 $(3)^3$ Orthogonal experimental results of degradation of Thiamethoxam by *S.maltophilia*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	25	6	1.0	56.540	35.4679	8.21	$\ln(C_t/C_o) = -128.57x + 59.714$	0.96
2	25	7	1.5	69.900	36.4051	5.91	$\ln(C_t/C_o) = -567.57x+199.35$	0.99
3	25	8	2.0	62.826	35.9513	7.16	$\ln(C_t/C_o)= - 71.429x + 25.5$	0.89
4	30	6	1.5	70.646	36.9514	5.75	$\ln(C_t/C_o) = -91.044x+ 22.378$	0.93
5	30	7	2.0	75.800	38.0050	4.88	$\ln(C_t/C_o) = 64.286x + 9.7857$	0.96
6	30	8	1.0	76.496	37.2299	6.42	$\ln(C_t/C_o)= -113.4x+ 22.959$	0.93
7	35	6	2.0	79.674	37.5694	4.44	$\ln(C_t/C_o) = 114.02x+19.556$	0.82
8	35	7	1.0	82.530	38.3018	4.04	$\ln(C_t/C_o)=-1285.7x+243.14$	0.96
9	35	8	1.5	73.510	37.7316	6.97	$\ln(C_t/C_o)= -1255.1x+387.85$	0.97

Table 4.38 The L_9 $(3)^4$ Orthogonal experimental results of degradation of Spiromesifen by *B. subtilis*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	30	6	1.5	72.79	37.2414	7.16	$\ln(C_t/C_o) = -7.9453x+12.871$	0.98
2	30	7	2.0	71.88	37.1322	7.36	$\ln(C_t/C_o) = -9.1154x + 13.419$	0.98
3	30	8	2.5	73.00	37.2665	7.56	$\ln(C_t/C_o) = -9.8704x + 12.676$	0.94
4	35	6	2.0	73.52	37.3281	6.97	$\ln(C_t/C_o) = -9.8778x + 12.624$	0.94
5	35	7	2.5	70.29	36.9379	5.75	$\ln(C_t/C_o) = -11.809 + 13.725$	0.97
6	35	8	1.5	71.29	37.0606	5.59	$\ln(C_t/C_o) = -12.475 + 14.014$	0.98
7	40	6	2.5	69.65	36.8584	5.46	$\ln(C_t/C_o) = -19.927x + 17.715$	0.97
8	40	7	1.5	65.21	36.2863	6.60	$\ln(C_t/C_o) = -15.064x + 15.798$	0.99
9	40	8	2.0	68.65	36.7328	6.08	$\ln(C_t/C_o) = -18.817 + 19.172$	0.98

Table 4.39 The $L_9 (3)^3$ Orthogonal experimental results of degradation of Spiromesifen by *S. geniculate*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	30	6	1.5	76.640	37.6891	6.42	$\ln(C_t/C_o) = -11.865 + 12.22$	0.95
2	30	7	2.0	82.680	38.3480	4.04	$\ln(C_t/C_o) = -10.29x + 10.918$	0.93
3	30	8	2.5	75.693	37.5811	4.98	$\ln(C_t/C_o) = -14.664x + 12.619$	0.87
4	35	6	2.0	82.570	38.3364	4.04	$\ln(C_t/C_o) = -17.593x + 12.98$	0.96
5	35	7	2.5	83.210	38.4035	3.91	$\ln(C_t/C_o) = -21.924x + 13.04$	0.95
6	35	8	1.5	81.840	38.2593	4.17	$\ln(C_t/C_o) = -142.27x + 38.58$	0.98
7	40	6	2.5	79.790	38.0390	4.32	$\ln(C_t/C_o) = -21.3x + 15.231$	0.99
8	40	7	1.5	81.740	38.2487	4.17	$\ln(C_t/C_o) = -33.189x + 15.937$	0.96
9	40	8	2.0	80.156	38.0747	4.30	$\ln(C_t/C_o) = -39.65x + 18.693$	0.98

Table 4.40 The L_9 $(3)^3$Orthogonal experimental results of degradation of propargite by *C. cucumeris*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	25	6	1.0	72.170	37.1671	5.44	$\ln(C_t/C_o) = -18.503x+13.734$	0.88
2	25	7	1.5	73.350	37.3080	5.29	$\ln(C_t/C_o) = -14.223x + 12.44$	0.97
3	25	8	2.0	69.230	36.8059	5.91	$\ln(C_t/C_o) = -25.822x + 16.433$	0.96
4	30	6	1.5	70.760	36.9958	6.00	$\ln(C_t/C_o) = -56.558x + 21.386$	0.93
5	30	7	2.0	71.008	37.0261	5.61	$\ln(C_t/C_o) = -97.85x + 32.263$	0.99
6	30	8	1.0	70.050	36.9082	5.75	$\ln(C_t/C_o) = -58.873x + 23.58$	0.90
7	35	6	2.0	73.080	37.2760	5.62	$\ln(C_t/C_o) = -62.552x + 21.446$	0.94
8	35	7	1.0	71.150	37.0435	5.60	$\ln(C_t/C_o) = -52.883x + 23.42$	0.88
9	35	8	1.5	74.150	37.4022	5.14	$\ln(C_t/C_o) = -57.86x + 20.265$	0.97

Table 4.41 The L_9 $(3)^3$ Orthogonal experimental results of degradation of glyphosate by *A. baumannii*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	25	6	1.5	79.877	38.0484	4.44	$\ln (C_t/C_o) = -46.112x + 20.139$	0.94
2	25	7	2.0	80.227	38.0864	4.30	$\ln (C_t/C_o) = -46.903x + 19.594$	0.91
3	25	8	2.5	75.680	37.5796	5.04	$\ln (C_t/C_o) = -57.11x + 23.957$	0.89
4	30	6	2.0	81.264	38.1980	4.17	$\ln (C_t/C_o) = -44.965x + 19.698$	0.92
5	30	7	2.5	78.040	37.8463	4.57	$\ln (C_t/C_o) = -48.247x + 20.62$	0.96
6	30	8	1.5	77.990	37.8408	4.71	$\ln (C_t/C_o) = -50.075x + 16.074$	0.91
7	35	6	2.5	82.500	38.3291	4.04	$\ln (C_t/C_o) = -35.782x + 16.074$	0.95
8	35	7	1.5	74.042	37.3896	6.99	$\ln (C_t/C_o) = -46.114x + 23.929$	0.94
9	35	8	2.0	83.020	38.3837	3.91	$\ln (C_t/C_o) = -23.263x + 13.145$	0.94

Table 4.42 The $L_9 (3)^3$ Orthogonal experimental results of degradation of ethion by *S.maltophilia*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	30	6	1.0	92.380	39.3116	2.74	$\ln (C_t/C_o) = -64.067x + 14.755$	0.93
2	30	7	1.5	93.34	39.4014	2.60	$\ln (C_t/C_o) = -49.878x + 12.029$	0.87
3	30	8	2.0	92.630	39.3350	2.73	$\ln (C_t/C_o) = -108.28x + 12.642$	0.90
4	35	6	1.5	94.080	39.4699	2.46	$\ln (C_t/C_o) = -57.61x + 12.167$	0.89
5	35	7	2.0	94.321	39.4922	2.53	$\ln (C_t/C_o) = -50.79x + 19.17$	0.96
6	35	8	1.0	92.655	39.3374	2.62	$\ln (C_t/C_o) = -54.025x + 15.53$	0.96
7	40	6	2.0	94.486	39.5073	2.41	$\ln (C_t/C_o) = -34.062x + 10.441$	0.92
8	40	7	1.0	93.641	39.4293	2.60	$\ln (C_t/C_o) = -34.725x + 10.616$	0.89
9	40	8	1.5	93.865	39.4501	2.58	$\ln (C_t/C_o) = -36.927x + 10.757$	0.88

Table 4.43 The $L_9\ (3)^3$ Orthogonal experimental results of degradation of deltamethrin by *S.maltophilia*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	$t_{1/2}$	Regression equation	R^2
1	30	6	1.5	96.57	39.6968	2.17	$\ln (C_t/C_o) = -35.481x + 10.42$	0.92
2	30	7	2.0	95.50	39.6001	2.31	$\ln (C_t/C_o) = -36.166x + 10.571$	0.90
3	30	8	2.5	96.01	39.6463	2.29	$\ln (C_t/C_o) = -34.299x + 10.179$	0.93
4	35	6	2.0	92.10	39.2852	2.74	$\ln (C_t/C_o) = -45.218x + 13.21$	0.88
5	35	7	2.5	94.28	39.4884	2.46	$\ln (C_t/C_o) = -42.915x + 12.13$	0.95
6	35	8	1.5	90.99	39.1799	3.01	$\ln (C_t/C_o) = -50.063x + 13.597$	0.92
7	40	6	2.5	91.19	39.1989	2.87	$\ln (C_t/C_o) = -50.246x + 13.637$	0.92
8	40	7	1.5	97.90	39.8157	1.99	$\ln (C_t/C_o) = -37.529x + 11.173$	0.95
9	40	8	2.0	93.65	39.4302	2.60	$\ln (C_t/C_o) = -36.927x + 10.757$	0.96

Table 4.44 The $L_9 (3)^3$ Orthogonal experimental results of degradation of quinalphos by P. alvei

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	T1/2	Regression equation	R^2
1	30	5	1.5	52.123	34.3406	9.44	$\ln (C_t/C_o) = -19.56x + 23.066$	0.99
2	30	6	2.0	58.903	35.4027	7.99	$\ln (C_t/C_o) = - 11.55x + 15.457$	0.89
3	30	7	2.5	58.366	35.3232	7.90	$\ln (C_t/C_o) = -12.763x + 16.58$	0.92
4	35	5	2.0	57.325	35.1669	8.21	$\ln (C_t/C_o) = - 16.269x + 19.894$	0.97
5	35	6	2.5	61.099	35.7207	7.3	$\ln (C_t/C_o) = -14.064x + 17.808$	0.94
6	35	7	1.5	60.947	35.6990	7.5	$\ln (C_t/C_o) = - 14.549x + 18.265$	0.95
7	40	5	2.5	52.820	34.4560	9.23	$\ln (C_t/C_o) = -20.547x + 24.037$	0.98
8	40	6	1.5	57.070	35.1282	8.21	$\ln (C_t/C_o) = -35.565x + 39.715$	0.90
9	40	7	2.0	63.198	36.0141	6.97	$\ln (C_t/C_o) = -12.887x + 16.834$	0.91

Table 4.45 The $L_9\ (3)^3$ Orthogonal experimental results of degradation of fenpyroximate by *P. alvei*

RUNS	Temp(1)	pH (2)	Cell size (3)	Deg (%)	S/N ratio	T1/2	Regression equation	R^2
1	30	5	1.5	76.17	34.3406	10.21	$\ln (C_t/C_o) = -16.56x+ 13.066$	0.94
2	30	6	2.0	80.103	35.4027	4.98	$\ln (C_t/C_o) = - 10.55x+ 19.457$	0.98
3	30	7	2.5	80.8	35.321	6.12	$\ln (C_t/C_o) = -18.763x+ 16.58$	0.96
4	35	5	2.0	80.577	35.1669	5.66	$\ln (C_t/C_o) = - 19.269x+ 16.894$	0.98
5	35	6	2.5	80.35	34.7207	7.7	$\ln (C_t/C_o) = -21.064x+ 13.707$	0.94
6	35	7	1.5	80.577	35.6990	8.5	$\ln (C_t/C_o) = -25.549x+ 18.268$	0.96
7	40	5	2.5	76.08	33.4570	6.25	$\ln (C_t/C_o) = -18.547x+ 14.036$	0.96
8	40	6	1.5	79.24	35.1000	6.12	$\ln (C_t/C_o) = -25.565x + 29.715$	0.91
9	40	7	2.0	80.23	35.0141	5.9	$\ln (C_t/C_o) = -10.887x+ 12.834$	0.94

Table No 4.46 : ANALYSIS OF VARIANCE

a. Analysis of Variance – P aeruginosa Fenpyroximate

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	0.7901	2.48%	0.7901	0.3950	3950.33	0.000
B	2	30.7945	96.55%	30.7945	15.3972	153972.33	0.000
C	2	0.3085	0.97%	0.3085	0.1542	1542.33	0.001
Error	2	0.0002	0.00%	0.0002	0.0001		
Total	8	31.8932	100.00%				

b. Analysis of Variance TXM

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	383.846	71.60%	383.846	191.923	5.63	0.151
B	2	81.049	15.12%	81.049	40.524	1.19	0.457
C	2	3.085	0.58%	3.085	1.543	0.05	0.957
Error	2	68.142	12.71%	68.142	34.071		
Total	8	536.122	100.00%				

c. Analysis of Variance spiromesifen by *Bacillus*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	37.9376	69.02%	37.9376	18.9688	143.69	0.007
B	2	12.6278	22.97%	12.6278	6.3139	47.83	0.020
C	2	4.1347	7.52%	4.1347	2.0673	15.66	0.060
Error	2	0.2640	0.48%	0.2640	0.1320		
Total	8	54.9642	100.00%				

d. Analysis of Variance spiromesifen

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	26.532	45.45%	26.532	13.266	6.43	0.135
B	2	19.469	33.35%	19.469	9.735	4.72	0.175
C	2	8.244	14.12%	8.244	4.122	2.00	0.334
Error	2	4.128	7.07%	4.128	2.064		
Total	8	58.373	100.00%				

e. Analysis of Variance glyphosate

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	2.411	3.30%	2.411	1.205	0.11	0.899
B	2	21.769	29.80%	21.769	10.885	1.01	0.497
C	2	27.348	37.43%	27.348	13.674	1.27	0.440
Error	2	21.529	29.47%	21.529	10.765		
Total	8	73.058	100.00%				

f. Analysis of Variance propargite

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	7.204	33.78%	7.204	3.6019	0.96	0.510
B	2	1.247	5.85%	1.247	0.6237	0.17	0.857
C	2	5.371	25.19%	5.371	2.6855	0.72	0.583
Error	2	7.503	35.18%	7.503	3.7514		
Total	8	21.325	100.00%				

g. Analysis of Variance ethion

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value
A	2	2.38239	48.70%	2.38239	1.19120	309.21
B	2	0.89088	18.21%	0.89088	0.44544	115.63
C	2	1.61122	32.93%	1.61122	0.80561	209.12
Error	2	0.00770	0.16%	0.00770	0.00385	
Total	8	4.89221	100.00%			

h. Analysis of Variance deltamethrin

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	19.117	39.55%	19.117	9.559	1.46	0.007
B	2	12.355	25.56%	12.355	6.178	0.94	0.015
C	2	3.735	7.73%	3.735	1.868	0.28	0.079
Error	2	13.131	27.16%	13.131	6.566		
Total	8	48.339	100.00%				

i. Analysis of Variance quinalphos

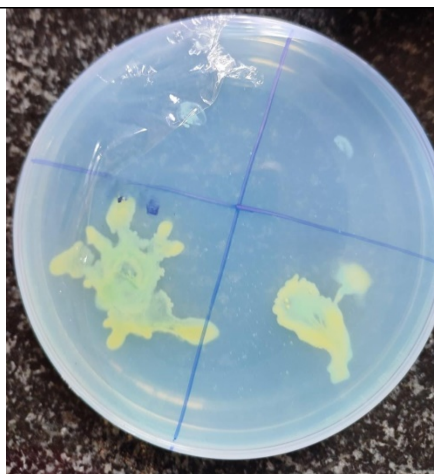
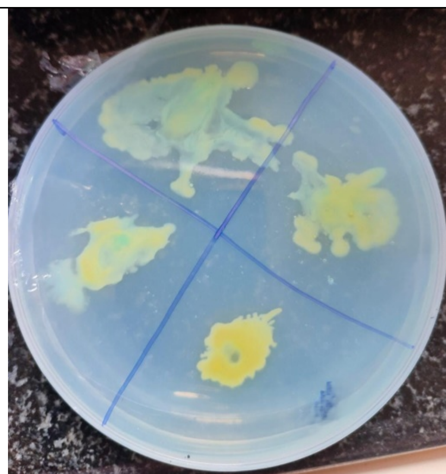
Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	16.969	15.58%	16.969	8.484	5.56	0.152
B	2	73.169	67.16%	73.169	36.584	23.98	0.040
C	2	15.758	14.46%	15.758	7.879	5.16	0.062
Error	2	3.051	2.80%	3.051	1.526		
Total	8	108.947	100.00%				

j. Analysis of Variance of fenpyroximate degradation by P.alvei

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
A	2	0.7973	2.50%	0.7973	0.3987	3916.91	0.000
B	2	30.8022	96.54%	30.8022	15.4011	151320.83	0.000
C	2	0.3063	0.96%	0.3063	0.1531	1504.67	0.001
Error	2	0.0002	0.00%	0.0002	0.0001		
Total	8	31.9060	100.00%				

4.4.3 Preparation of bacterial consortia and degradation of deltamethrin by using the consortium in liquid MSM

Two bacterial consortia were created using the ten bacterial strains isolated from the tea garden soils that were identified earlier. These consortia were formulated based on the co-habitation assay. The tea plantation isolates were examined for co-habitation for the formulation of microbial consortia for the breakdown of deltamethrin. It is crucial to assess the co-survival capability of soil isolates from tea gardens for the development of microbial consortia. Consortia based studies highlight the significance of establishing the co-existence of individual isolates at the primary level. The soil isolates from the native tea plantation were inoculated in various combinations to assess their co-survival with one another. The co-habitation study revealed the development of a zone of inhibition between specific isolates after the introduction and incubation of various tea plantation soil isolates. The tea plantation isolates DRNB1, SFN1, and FXE1 exhibited compatibility and showed no antagonistic effects when grown together. The isolates GLYB2, PTEB2, Q1T, F1T, and SFT1 exhibited mutual tolerance and were able to grow together successfully without any antagonistic effects. The observation of overlapping between bacterial colonies at the contact point clearly indicates growth compatibility without any antagonism in both sets of bacterial isolates. Consequently, two microbial consortia were formulated and named as Pesticide Degrading Microbial Consortium 1 (PDMC1), and Pesticide Degrading Microbial Consortium 2 (PDMC2). The bacterial sp. *Stenotrophomoas maltophilia* strain DRNB1, *Stenotrophomonas geniculata* strain SFN1, and *Pseudomonas aeruginosa* strain FXE1constitute the consortium PDMC1. The consortium PDMC2 is formulated by the isolates *Acinetobacter baumannii, Chryseobacterium cucumeris, Bacillus subtilis, and Paenibacillus alvei.* The present work focused on the degrading efficiency of PDMC1 among the two microbial consortia. Deltamethrin was chosen as the insecticide for the microbial consortia studies. Deltamethrin has become very prevalent due to its extensive application in agricultural fields and tea plantations in Anamalai Hills. Therefore, the degradation of deltamethrin by microbial consortium was analysed in the present study. The parameters chosen for the breakdown of deltamethrin by microbial consortium PDMC1 were temperature of 35°C, pH range of 6 to 7, and a bacterial inoculum size of 1 (OD @ 600nm). The pesticide concentration was determined quantitaviely based on GC-MS analysis. The degradation percentage was calculated by using the equation no (5) illustrated in chapter 3 section 3.9.5.

  PDMC2	 PDMC1
 SET OF FOUR STRAINS SHOWING ANTAGONISM (DRNB1, Q1T, F1T,and GLYB2)	 SET OF FOUR STRAINS SHOWING ANTAGONISM
 STRAINS SHOWING CO-HABILITATION (DRNB1, TXM2, EON2, and SFN1)	 STRAINS SHOWING CO-HABILITATION

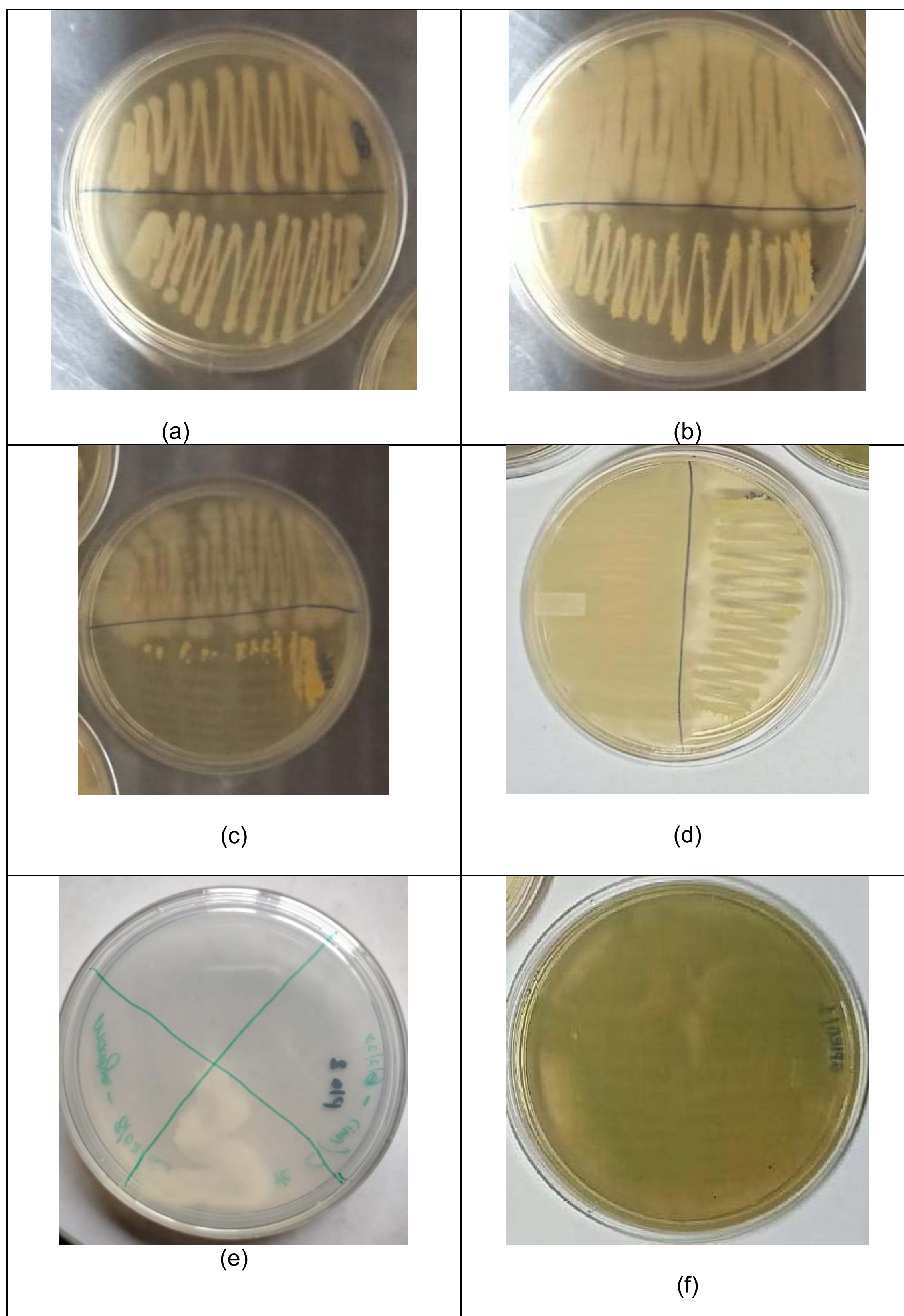


Figure No 4.67 Co-Habitation studies for consortia formulation. (a)-DRNB1 & GLYB2, (b) TXM2 & PTEB2, (c) and (d) EON2 & Q1T & F1T, (e)&(f) SFN1 showing antagonism with Q1T.

The strains DRNB1, SFN1, and FXE1 were individually examined for their ability to use and subsequently degrade deltamethrin at an initial concentration of 100 ppm in liquid MSM. After a period of 10 days, it was observed that the three strains exhibited degradation of deltamethrin, with degradation rates ranging from 58.6% to 96.2%. After a five-day incubation period, strain DRNB1 shown more efficiency compared to the other two strains. DRNB1 utilised 60.2% of deltamethrin, whereas strains FXE1 and SFN1 were able to degrade 42.1% and 28% of deltamethrin respectively after five days.

When liquid MSM was inoculated with strain DRNB1, the degradation of 26.9% was detected on the third day. This means that the initial concentration of 100ppm was reduced to 73.1 ppm. The remaining amount was further decreased to 39.8 ppm during a span of five days, and to 9.8 ppm within eight days. The overall degradation of deltamethrin reached 96.2% within a period of 10 days, beginning with an initial deltamethrin concentration of 100 ppm.

When liquid MSM was treated with strain SFN1, a degradation of 16% was detected on the third day. This implies that the initial concentration of 100 ppm was reduced to 84 ppm. The remaining amount was further reduced to 72 ppm within five days, and 59 ppm within a span of eight days. After 10 days, the total degradation of deltamethrin amounted to 58.6%, starting with an initial deltamethrin level of 100 ppm.

When liquid MSM was inoculated with strain FXE1, a degradation of 29.6% was observed on the third day. Thus, the initial concentration of 100 ppm declined to 70.4 ppm. the remaining amount was further decreased to 57.9 ppm within a span of five days, and to 31 ppm within eight days. The overall degradation of deltamethrin reached 73.6% after 10 days, starting with an initial concentration of 100 ppm.

When liquid MSM was inoculated with a combination (PDMC1) of DRNB1, FXE1, and SFN1 cultures, which had an initial deltamethrin level of 100 ppm, a degradation of 45.8% was observed after 24 hours of incubation. On the third day, there was a 60.1% decrease in the concentration of deltamethrin, resulting in a remaining value of 39.9 ppm from the original value. The residual quantity was subsequently decreased to 13.9 ppm during five days and further lowered to 6.9 ppm within eight days. The degradation of deltamethrin reached a level of 92.5% during a period of merely seven

days. After a 10 day incubation period, the degradation of deltamethrin achieved an overall rate of 96.5%.

The deltamethrin in the uninoculated control sample exhibited slow degradation in a linear manner, resulting in only 10.6% degradation after 10 days of incubation. Therefore, a concentration of 89.4 ppm of deltamethrin remained in the liquid MSM. Among the three isolates, *Stenotrophomonas maltophilia* strain DRNB1 demonstrated the highest degradation ability, with a degradation rate of 60.2% after five days, which increased to 96.2% after 10 days. *Pseudomonas aeruginosa* strain FXE1 also exhibited a moderate degradation ability, by removing 73.6% of deltamethrin after 10 days. The lowest degradation was observed in the MSM inoculated with *Stenotrophomonas geniculata* strain SFN1, with a degradation rate of 58.6% after 10 days of incubation.

Multiple studies have demonstrated the significance of bacterial consortia in collectively metabolizing hazardous substances (Field et al., 1995; Saratale et al., 2010; Zafra et al., 2014; Villaverde et al., 2017). The consortium PDMC1 effectively degraded 90.6% of the initial deltamethrin (100ppm) within a span of six days. On the other hand, when the isolates FXE1, SFN1, and DRNB1 were inoculated individually, only 58%, 32%, and 81.6% degradation of deltamethrin were observed during the same incubation time, respectively. This could be attributed to the physiological interconnection and mutual adaptability among the members of the consortium, enabling them to withstand the adverse effects of the hazardous substances (Abdulsalam et al., 2011; Pino and Penuela, 2011; Ahmad et al., 2018). The degradation rates were lower when inoculated with individual pure isolates separately compared to the microbial consortium. Optimal degradation may not be attained by the use of a single bacterial strain (Krishana and Philip, 2008). In this study, deltamethrin achieved a degradation of 90.6% in liquid MSM in the presence of microbial consortium within a period of 6 days. The highest rate of degradation occurred rapidly, in the presence of consortium, while the individual strains metabolised the deltamethrin only in the range of 32% to 81.6% during the same period. The utilisation of microbial consortium improved the degradation of deltamethrin by reducing the time for deterioration. Bacterial consortia possess a greater capacity to co-metabolize hazardous compounds compared to individual bacterial strains due to the presence of diverse bacterial species within the consortium,

which can express a variety of enzymes to facilitate the breakdown of pollutants (Hansen et al., 2001; Nestler et al., 2001). Researchers have discovered that pesticide-degrading bacterial consortia can cause improved and efficient breakdown of several persistent pollutants. For example, Sorensen et al., (2008) created a two-member consortium composed of *Arthrobacter globiformis* and *Cariovorax* sp. for the purpose of metabolizing diuron. Vidya Lakshmi et al., (2009) developed a consortium of aerobic bacteria consisting of *Pseudomonas aeruginosa, Bacillus cereus, Klebsiella sp., and Serratia marscenes.* This consortium was able to break down 80% to 84% of chlorpyrifos in liquid MSM. Dehghani et al., (2013) also established a consortia capable of degrading 60.5% of atrazine in soil samples. In a study conducted by Jabeen et al., (2015) a bacterial consortium was developed consisting of *Achromobacter xylosoxidans, P. aeruginosa, Bacillus sp.,* and *Citrobacter koseri* that degraded 93.39% of profenofos at pH 6.8 and temperature 35°C. Ahmad et al., (2018) developed a new bacterial consortium BDAM, which included *Achromobacter xylosoxidans, Achromobacter pulmonis,* and *Ochrobactrum intermedium*. This consortium degraded 97% of bispyribac sodium within a period of 21 days. Jariyal et al., (2018) generated microbial consortia composed of three microorganisms: *Brevibacterium sp, Bacillus sp,* and *Pseudomonas fulva*. These consortia demonstrated a remarkable capability to degrade phorate, with degradation rates ranging from 97.5% to 98.31% in liquid MSM. Gongora et al., (2020) studied the degradation of atrazine, carbofuran, and glyphosate by a microbial consortium consisting of 21 bacteria, where the prominent bacteria sp. was *Pseudomonas nitroreducens*.

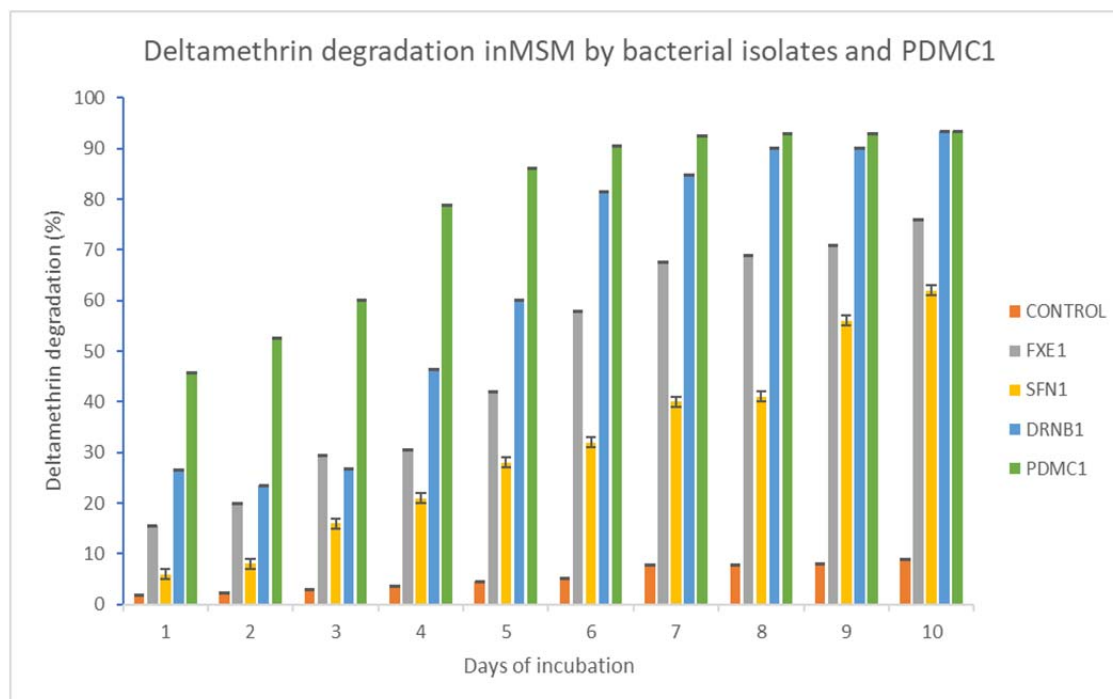


Figure 4.68: Deltamethrin degradation by microbial consortium. Values are expressed in Mean ± SD

One significant feature of the consortium of PDMC1 is its inclusion of bacteria from metabolically active and diverse genera, such as *Pseudomonas* and *Stenotrophomonas.* In addition, bacterial strains from this genus are identified for their ability to break down a wide range of pyrethroids insecticides, as well as other classes of pesticides including cypermethrin (Gur et al., 2014), fenpropathrin (Song et al., 2015), diazinon (Essa et al., 2016), propiconazole (Satapute and Kaliwal, 2016), chlorimuron-ethyl (Zang et al., 2016), endosulfan (Ozdal et al., 2017), cypermethrin (Tang et al., 2017; Gurjar and Hamde, 2018), and diazinon (Pourbabaee et al., 2018). In the present study, the *Stenotrophomonas maltophilia* strain DRNB1 demonstrated a high rate of deltamethrin elimination in liquid MSM with a degradation value of 96.2% within 10 days. This degradation value is comparable to that achieved by the bacterial consortium PDMC1. Wu et al., (2021) had previously shown the potential of *Stenotrophomonas maltophilia* strain XQ08 to degrade deltamethrin in contaminated sites.

Studies on deltamethrin degradation by microbial consortia are very limited. Cycon et al., (2014) showed that two strains of *Serratia marcescens* were able to break down deltamethrin in soil samples. They found that these strains degraded between 82.8% to 88.3% of deltamethrin. Tang et al., (2020) demonstrated the breakdown of

deltamethrin using a co-culture of *Acinetobacter junii LH-1-1* and *Klebsiella pneumoniae* BPBA052. The presence of this bacterial co-culture significantly increased the breakdown of deltamethrin, resulting in a degradation efficiency of 94.25%.

The outcomes of the aforementioned studies and that of the present study indicate that the bacterial consortium PDMC1 was successful in degrading deltamethrin. This consortium has the potential to enhance the existing group of microorganisms used for the remediation of regions contaminated with pyrethroid and other pesticides.

5. SUMMARY AND CONCLUSION

Agrochemicals (synthetic fertilizers and insecticides) have been used on agricultural fields for several decades to ensure sustainable agricultural output. The development of insecticides for agricultural use was prompted by concerns about crop loss from pest infestation. Due to their potent insecticidal activity, relative lack of persistence (as opposed to chlorinated pesticides), and financial advantages , pesticides are widely used across the world to combat pest infestation. Pesticides accumulate in high quantities in the environment due to continuous and unchecked field application at concentrations above authorized dosages, which causes bioaccumulation. Among the pesticides applied, only 1% reaches the target pest, while the remaining pesticide residue will settle in the environment. Pesticide residues over the maximum residual limit (MRL) have been found in numerous samples of soil, water, air, food, dairy products, and blood. There is evidence that non-target plants and animals are in peril as a result of persistent pesticide residues. By affecting the physical, chemical, and biological structure of the soil, pesticide residues in agricultural soil have an impact on soil fertility. consequentially, it becomes vital to get rid of the lingering pesticide residues found in agricultural soils.

The three most popular processes—photolysis, chemical degradation, and biodegradation— generally carry out the majority of the degradation of pesticides. Among the three methods, biodegradation is regarded as the most secure, affordable, and environmentally beneficial method for completely mineralizing organic contaminants like pesticides. The main goal of the thesis is to create an approach that would break down pesticide residues with the use of native tea garden soil bacterial isolates and to prepare a microbial consortium for the degradation of pesticides.

In 2017, a pilot study was carried out in Anamalai Hills, Tamil Nadu. The purpose was to gather information from scientists and specialists at the UPASI Tea Research Foundation in Valparai regarding the use of pesticides in the plantation areas, and to determine the specific study sites for soil sample collection. The pilot study provided valuable insights into the existing literature and methodological advancements in the field of pesticide degradation studies at Anamalai Hills, as well as their relevance to the current study.

The soil samples were collected from eight different sites named S1 to S8, including forest soil (Virgin soil), two sites where natural control against pests are used, and the

transition area between the forest and estates. The physicochemical properties of all the soil samples were analysed (pH, Electrical conductivity, organic carbon, N,P,K, moisture content, and micronutrients). The bacterial population in all the soil samples during the three seasons (pre-monsoon, monsoon, and post-monsoon) was enumerated during one year 2018-2019. The pesticide residue analysis of all the soil samples was done, and the residue level was below the maximum residue level.

The pesticides selected in the study were deltamethrin, ethion, thiamethoxam, spiromesifen, propargite, quinalphox, fenpyroximate, and glyphosate. The isolation of natural microorganisms from pesticide-challenged agricultural fields was carried out in the study. To perform experiments on pesticide breakdown, a total of ten bacterial isolates were chosen based on their development under chemical stress. The selected bacterial isolates were named with abbreviations of their respective pesticides DRNB1, EON2, TXM1, SFN1, F1T, Q1T, GLYB2, PTEB2, FXE1, and SFT1. The isolated bacterial strains were characterized morphologically and phenotypically, and molecular characterization was done at last for the species level identification. *Stenotrophomonas maltophilia, Paenibacillus alvei, Stenotrophomonas geniculata, Bacillus subtillis, Pseudomonas aeruginosa, Chryseobacterium cucumeris,* and *Acinetobacter baumannii* were identified as the native tea garden isolates using biochemical and molecular investigation. The growth of bacterial isolates at different nitrogen and carbon sources along with pesticides was studied. All of the bacterial strains exhibited different patterns of growth in different carbon and nitrogen sources. The biosurfactant production and biofilm formation of all the bacterial strains were tested. The esterase activity of all the isolates was also studied. The disc diffusion assay was used to test the maximum level of tolerance or resistance of the bacterial isolates toward different concentrations of pesticides.

The degradation of each pesticide at different parameters was studied. The parameters selected were pH, temperature, and bacterial inoculum size. The half-life and the degradation constant are also calculated. The degradation at different parameters was done in Mineral media (MSM). On the basis, of One -way ANOVA the best three values for each parameter were identified and degradation studies were again done for the optimization study. The optimization of parameters temperature, pH, and inoculum size was done by using the Taguchi optimization Methodology. After the optimization process, the degradation of pesticides in soil samples was done for the

period of three months. Metabolites produced by the degradation process of pesticides were identified using GC-MS analysis. A co-habitation assay was done for the preparation of microbial consortium, to identify the synergetic and antagonistic effects between the bacterial strains. A microbial consortium (PDMC1-Pesticide Degrading Microbial Consortium) was created for the degradation of the pesticide deltamethrin, and about 91% of the pesticide degradation in MSM was observed. DRNB1-*Stenotrophomonas maltophilia*, SFN1-*Stenotrophomonas [Pseudomonas] geniculata*, and FXE1-*Pseudomonas aeruginosa* are the three bacterial isolates that make up the consortium.

The outcome obtained from the present study is explained below:

Conclusion 1: The physicochemical parameters such as soil pH, soil nitrogen, soil organic carbon, soil phosphorous, soil potassium, soil electrical conductivity, soil texture, and micronutrients like zinc, iron, manganese, and copper were analysed during three different seasons the physicochemical parameters of the soil are highly correlated with each other.

Even though acidic soils are ideal for the growth of tea and coffee, it is necessary to maintain the soil pH at the optimum level. It also helps to conserve the soil microbial community and soil enzyme activities. the soil moisture content rises after the monsoon, progressively falls during the post-monsoon, and displays low values before the monsoon. the EC of the soil is dependent on the temperature, water content, and application of pesticides. The seasonal variations highly affected the soil organic carbon and soil nitrogen. Microbial activity also enhances phosphorous availability in soil. Soil texture influences the retention rate of pesticides in soils. The soil parameters influence the stability of the microbial community and the pesticide fate in the environment. The seasonal variations also affect the soil parameters, microbial population, and the behaviour of pesticides in the environment. The soil texture plays a significant role in the degradation and absorption of pesticides in the environment.

Conclusion 2: the bacterial strains with the ability to degrade all the pesticides were isolated and characterized up to the molecular level. The bacteria isolated were *Stenotrophomonas maltophilia* strain DRNB1, EON2, and TXM1 that degrades deltamethrin, ethion, and thiamethoxam respectively. *Pseudomonas aeruginosa* strain FXE1 degrades fenpyroximate, spiromesifen degrading two strains *Paenibacillus*

alvei, and *Stenotrophomonas geniculata*. *Bacillus subtilis* and *Chryseobacterium cucumeris* strain that degrade glyphosate and propargite. Finally, quinalphos degrading bacteria *Paenibacillus alvei* strain Q1T and their biosurfactant and biofilm formation were also studied. The bacterial strain has high biosurfactant and biofilm formation, and the strain with minimum biosurfactant and biofilm formation was exhibited by *Chryseobacterium cucumeris*.

Conclusion 3: the optimization of parameters for the degradation of each pesticide and bacterial strain was done by Taguchi analysis. Three different pH, temperature, and inoculum sizes for all the sets of degradation were studied on the basis of S/N ratios. The growth of bacterial strains at different carbon and nitrogen sources was analyzed. The bacterial strains preferred carbon sources, and certain bacteria exhibited high growth in nitrogen sources.

Conclusion 4: two microbial consortia was prepared, PDMC1 and PDMC2. The degradation of deltamethrin by PDMC1 was studied. The deltamethrin was degraded up to 96.5% in the presence of PDMC1. The microbial consortium in PDMC1 was *S.maltophilia* strain DRNB1, *S.geniculata* Strain SFN1, and *Pseudomonas aeruginosa* strain FXE1

Challenges faced during the study.

- The continual exposure to pesticides during experimental investigations resulted in significant health complications such as throat infections and allergies. This caused a delay in the laboratory studies due the medical advice and treatments.
- As a result of fluctuations in the climatic conditions at Anaimali Hills, the regular visits to study sites and the collecting of soil samples from these sites became time-consuming, leading to a progressive delay in the development of the research work.
- The covid-19 pandemic lockdown disrupted both the process of collecting soil samples and the experiments in the laboratory. This also resulted in the contamination of collected and cultured bacterial samples in the laboratory.
- The unexpected microbiological contamination occurring at times during the experimental period necessitated the repetition of the trials.

- The soil samples were collected through the collaborative efforts between officials at UPASI Tea Research Foundation at Anaimalai Hills. However, acquiring permission from the higher officials in the estate areas to collect soil samples from the study sites was a complicated process.

LIMITATAIONS OF THE STUDY

- The research focused only on limited geographical area
- The research studied only the degradation of certain kind of pesticides.
- The study was done only in laboratory conditions and not in field conditions.

6. RECOMMENDATIONS

In the framework of the present study, the following recommendations are proposed.

- Spiromesifen and Fenpyroximate are advanced acaricides that are currently prevalent in plantations and agricultural fields due to their exceptional effectiveness in pest management. However, the extensive application of these acaricides has a detrimental impact on natural resources. There is a dearth of research on the degradation of both these acaricides. This investigation focused on the isolation and examination of the breakdown of these acaricides by three different bacterial species. The outcome of this study would serve as a resourceful platform for further comprehensive research in identifying superior and distinct bacterial strains in the environment that can degrade these acaricides.
- The study focused on examining bacterial growth in various carbon and nitrogen sources. Therefore, it paves the way for examining the breakdown of pesticides when carbon and nitrogen sources are added. It is recommended to investigate the impact of adding carbon and nitrogen sources at the rate at which pesticides are metabolised through bacterial degradation.
- The study mainly dealt with optimising parameters such as temperature, pH and bacterial inoculum size in order to determine the maximum level of pesticide degradation. To achieve the highest pesticide removal from contaminated sites it is recommended to optimise additional crucial factors such as incubation time, shaker rpm, and concentration of the pesticide.
- The investigation examined the synthesis of biosurfactant and biofilm formation characteristics of the bacterial isolates at a preliminary level, utilising the Tube method, CTAB test, and Congo Red agar method. However, the rate of biodegradation may be improved by conducting an extensive examination of biosurfactant and biofilm features of the bacterial strains at molecular level.
- The study demonstrated the degradation of deltamethrin by the Pesticide Degrading Microbial Consortium 1 (PDMC1) consortia in liquid minimal media out of the two microbial consortia prepared, PDMC1 and PDMC2. This may be extended to investigate the pesticide degradation efficiency of consortium PDMC2 across various types of pesticides including deltamethrin. Additionally, the current investigation examined the deterioration of

deltamethrin by PDMC1 in liquid minimal media. Further studies may explore the degradation of deltamethrin and other classes of pesticides by PDMC1 in soil samples. After evaluating the pathogenic properties of the bacterial consortia, it is advisable to provide farmers with the combined bacteria formulation as a bioweapon to combat pesticides.

- The study concentrated on the breakdown of individual pesticides by bacterial isolates. However, it has been observed that the soil samples collected from plantation areas reveals the presence of various kinds of pesticides. It is necessary to eliminate all the pesticides simultaneously using bacterial isolates. Therefore, it is recommended to evaluate the ability of the bacterial strains obtained in the present study to simultaneously degrade a variety of pesticides, as this enhances the efficiency of the degradation process.
- It is recommended to examine the PGPR characteristics of the isolated bacterial species to enhance the growth of tea plants and facilitate the breakdown of the pesticides.

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APPENDIX

Coliform count

Coliform count was compared between season and also between dilution. Interaction between season and dilution was also tested. For this two-way ANOVA was carried out and the pair wise comparison was done by using leas significant difference (LSD) test. Results along with mean and standard error of the estimate

Table 1. Result of comparison of bacterial count between season and also between dilution

	Mean ± SE			
Dilution	Pre-monsoon	Monsoon	Post Monsoon	Overall dilution
10^5	67.63 ± 6.56^{bB}	56.63 ± 6.79^{bB}	116.33 ± 11.45^{a}	80.19 ± 5.77^{B}
10^6	93.92 ± 8.08^{bA}	125.25 ± 11.53^{aA}	103.88 ± 9.66^{ab}	107.68 ± 5.82^{A}
Overall season	80.77 ± 5.50^{b}	90.94 ± 8.30^{b}	110.1 ± 7.46^{a}	93.94 ± 4.24
F-value between season = 5.203**; P-value = 0.007 F-value between dilution = 13.289**; P-value < 0.001 F-value for interaction between dilution and season = 9.643**; P-value < 0.001				

** Significant at 0.01 level
Means having different small letter as superscript differ significantly within a raw (between season)
Means having different capital letter as superscript differ significantly within a column (between dilution)

Table 1 shows that all the F-values was found to be significant at 0.01 level as the P-values are less than 0.01. Significant F-value (5.203) for season indicates that there exists significant difference in coliform count between season. Pair wise comparison using LSD test in the case of between overall season averaged over two dilution shows that coliform count is significantly higher in post monsoon season compared to other two season and no significant difference was noted in coliform count in pre-monsoon and monsoon seasons.

Significant F-value (13.289) for dilution indicates that there exists significant difference in coliform count between dilution. Pair wise comparison using LSD test in the case of overall dilution averaged over all seasons shows that coliform count is significantly higher in 10^6 dilutions compared to 10^5 dilutions.

Interaction between season and dilution (F-value = 9.643) was also found to be significant indicating that the season variation is not same in two different dilution

and between dilution variation is not same in different seasons. Hence, pair wise comparison was done between season separately for each dilution and between dilution separately for each season. Results shows that the variation between season in 10^5 dilution is same as that observed in overall season. That is coliform count is significantly higher in post monsoon season compared to other two season and no significant difference was noted in coliform count in pre-monsoon and monsoon seasons. However, in the case of 10^6 dilution, coliform count is higher in monsoon season it shows no significant difference with post monsoon season. In this case significant difference was noted only between monsoon season and pre-monsoon season. Pairwise comparison between dilution in each season reveals that there exists significant difference in coliform count between dilution in pre-monsoon and monsoon season with 10^6 dilution having highest count compared to other dilution. No significant difference in coliform count was noted between dilution in post monsoon season.

Nutrient parameters

Between season variation in nutrient parameters in the soil of Valparai region was done by using one-way ANOVA followed by Duncan Multiple range tests (DMRT).

Table 2. Results of comparison of Nutrient contents between season in the soil of Valparai region

Variable	Pre-monsoon	Monsoon	Post Monsoon	F-value (P-value)
Organic Carbon	4.11 ± 0.18^{c}	5.84 ± 0.15^{a}	5.09 ± 0.13^{b}	27.828** (<0.01)
Nitrogen	406.81 ± 10.81^{b}	354.54 ± 8.79^{c}	438.92 ± 8.28^{a}	23.835** (<0.01)
Phosphorous	33.96 ± 0.74^{c}	42.37 ± 0.61^{a}	38.21 ± 0.55^{b}	39.300** (<0.01)
Potassium	297.16 ± 7.81	299.01 ± 7.58	300.36 ± 5.85	0.050^{ns} (0.952)

** Significant at 0.01 level; ns non-significant
Means having different small letter as superscript differ significantly within a raw (between season)

As the p-value in the case of Potassium is greater than 0.05, it can be inferred that there exists no significant difference in soil potassium between season. For all

other parameters, F-value is less than 0.01 which shows that there exists significant difference in the parameters organic carbon, nitrogen and phosphorous between season. Results of DMRT shows that these parameters are significantly different between all season. In the case of organic carbon, significantly higher value was observed in Monsoon season and significantly lower value was observed in pre-monsoon season. Phosphorous is also significantly higher in monsoon season and significantly lower in pre-monsoon season. However, Nitrogen content is significantly higher in post monsoon season and significantly lower in monsoon season.

Physio chemical parameters

Comparison of physiochemical parameters was also done by using one-way ANOVA followed by DMRT. The results of the same is given in Table 3.

Table 3. Results of comparison of physiochemical parameters between season in the soil of Valparai region

Variable	Pre-monsoon	Monsoon	Post Monsoon	F-value (P-value)
pH	5.01 ± 0.07^{c}	5.55 ± 0.06^{b}	6.00 ± 0.05^{a}	69.709** (<0.01)
Moisture content	38.32 ± 0.96^{c}	53.28 ± 0.87^{a}	46.16 ± 0.89^{b}	57.376** (<0.01)
Electrical conductivity	0.58 ± 0.02^{a}	0.39 ± 0.02^{c}	0.45 ± 0.01^{b}	31.041** (<0.01)

** Significant at 0.01 level
Means having different small letter as superscript differ significantly within a raw (between season)

In the case of all physiochemical parameters, p-value was found to be less than 0.01 indicating that there exists significant difference between season in these parameters. Results of DMRT shows that pH, Moisture content and Electrical conductivity varies significantly between all seasons. pH was significantly higher in post-monsoon season and significantly lower in pre-monsoon season. Moisture content was significantly higher in monsoon season and significantly lower in pre-monsoon season. Electrical conductivity was significantly higher in pre-monsoon season and significantly lower in monsoon season.

Pesticide residue

Comparison of pesticide residue in the soil was also done by using Kruskal Walli's ANOVA. The results of the same is given in Table 4.

Table 4. Results of comparison of pesticide residue between season in the soil of Valparai region

Pesticide	Pre-monsoon	Monsoon	Post Monsoon	χ^2-value (P-value)
Quinalphos	0.019 ± 0.012	0.047 ± 0.031	0.028 ± 0.018	0.138^{ns} (0.933)
Thiamethoxam	0.003 ± 0.003	0.03 ± 0.03	0.015 ± 0.015	0.015^{ns} (0.992)
Deltamethrin	0.016 ± 0.016	0.089 ± 0.089	0.063 ± 0.063	0.015^{ns} (0.992)
Propargite	0.055 ± 0.036	0.076 ± 0.051	0.064 ± 0.043	0.035^{ns} (0.983)
Glyphosate	0.021 ± 0.021	0.033 ± 0.033	0.025 ± 0.025	0.015^{ns} (0.992)
Fenpyroximate	0.021 ± 0.015	0.056 ± 0.038	0.041 ± 0.029	0.078^{ns} (0.962)
Spiromesifen	0.003 ± 0.003	0.025 ± 0.025	0.018 ± 0.018	0.015^{ns} (0.992)
Ethion	0.022 ± 0.022	0.048 ± 0.048	0.02 ± 0.02	0.015^{ns} (0.992)
ns non-significant				

In all cases, P-value is greater than 0.05. So there exists no significant difference in the pesticide residue in the soil between season.

Relation between bacterial count and soil parameters

For finding out the relation between bacterial count and soil parameters, Karl Pearson's correlation coefficient was worked and tested for its significance.

Table 5. Relation between nutrient parameters and coliform count

Parameters	Coliform count in 10^5 dilution	Coliform count in 10^6 dilution
Organic Carbon	0.331^{ns}	0.513**
Nitrogen	0.609**	0.429*
Phosphorous	0.033^{ns}	0.161^{ns}
Potassium	0.406*	.493**

** Significant at 0.01 level; * Significant at 0.05 level
ns Non-Significant

Table 6. Relation between physio-chemical parameters and coliform count

Parameters	Coliform count in 10^5 dilution	Coliform count in 10^6 dilution
pH	0.078^{ns}	-0.079^{ns}
Moisture Content	0.556**	0.784**
Electrical conductivity	0.438*	0.420*

** Significant at 0.01 level; * Significant at 0.05 level
ns non-significant

Table 7. Relation between soil texture parameters and coliform count

Parameters	Coliform count in 10^5 dilution	Coliform count in 10^6 dilution
Clay	0.593^{ns}	0.595^{ns}
Slit	0.588^{ns}	0.639^{ns}
Sand	-0.631^{ns}	-0.652^{ns}

ns Non-Significant

Relation of soil texture on soil nutrient parameters

Table 8. Relation between soil texture parameters and soil nutrient parameters

Soil nutrient Parameters	Soil texture parameters		
	Clay	Silt	Sand
Organic Carbon	0.111^{ns}	0.554^{ns}	-0.291^{ns}
Nitrogen	0.372^{ns}	0.618^{ns}	-0.493^{ns}
Phosphorous	0.138^{ns}	0.070^{ns}	-0.121^{ns}
Potassium	0.914**	0.886**	-0.965**

** Significant at 0.01 level; ns non-Significant

Relation of soil texture on soil physiochemical parameters

Table 9. Relation between soil texture parameters and soil physiochemical parameters

Soil physiochemical Parameters	Soil texture parameters		
	Clay	Silt	Sand
pH	-0.198^{ns}	-0.435^{ns}	0.304^{ns}
Moisture Content	0.443^{ns}	0.258^{ns}	-0.401^{ns}
Electrical conductivity	0.373^{ns}	0.145^{ns}	-0.309^{ns}

ns non-Significant

Relation between pesticide residue with bacterial count and soil parameters

For finding out the relation between bacterial count and soil parameters, Spearman's Rank correlation coefficient was worked and tested for its significance.

Table 10. Relation of pesticide residue with bacterial count

Pesticide residue	Coliform count in 10^5 dilution	Coliform count in 10^6 dilution
Quinalphos	-0.085	-0.106
Thiamethoxam	-0.384*	-0.242
Deltamethrin	-0.242	-0.386*
Propargite	-0.520**	-0.451**
Glyphosate	-0.313	-0.342
Fenpyroximate	-0.080	-0.141
Spiromesifen	-0.304	-0.344
Ethion	-0.249	-0.388*

** Significant at 0.01 level; * Significant at 0.05 level

Table 11. Relation between nutrient parameters and pesticide residue

Parameters	Organic carbon	Nitrogen	Phosphorous	Potassium
Quinalphos	0.131	-0.022	0.12	-0.267
Thiamethoxam	0.205	-0.228	-0.504**	-0.394*
Deltamethrin	0.125	0.407*	0.172	0.066
Propargite	0.082	-0.270	-0.189	0.089
Glyphosate	-0.062	-0.137	0.171	0.411*
Fenpyroximate	0.129	-0.007	0.127	-0.316
Spiromesifen	-0.065	-0.136	0.168	0.409*
Ethion	0.123	0.405*	0.159	0.062

** Significant at 0.01 level; * Significant at 0.05 level

Table 12. Relation between physio-chemical parameters and pesticide residue

Parameters	pH	Moisture Content	Electrical conductivity
Quinalphos	-0.274	-0.218	-0.442*
Thiamethoxam	0.341	-0.282	-0.240
Deltamethrin	-0.324	-0.211	-0.402*
Propargite	0.154	-0.410*	-0.219
Glyphosate	-0.085	-0.262	-0.070
Fenpyroximate	-0.266	-0.224	-0.427*
Spiromesifen	-0.080	-0.266	-0.070
Ethion	-0.329	-0.209	-0.395*

* Significant at 0.05 level

Table 13. Relation between soil texture parameters and pesticide residue

Parameters	Clay	Silt	sand
Quinalphos	-0.232^{ns}	-0.349^{ns}	0.281^{ns}
Thiamethoxam	-0.439^{ns}	-0.176^{ns}	0.425^{ns}
Deltamethrin	0^{ns}	-0.176^{ns}	0.085^{ns}
Propargite	-0.291^{ns}	0.257^{ns}	-0.008^{ns}
Glyphosate	0^{ns}	0.439^{ns}	-0.340^{ns}
Fenpyroximate	-0.232^{ns}	-0.349^{ns}	0.281^{ns}
Spiromesifen	0^{ns}	0.439^{ns}	-0.340^{ns}
Ethion	0^{ns}	-0.176^{ns}	0.085^{ns}

ns non-significant

[GC-2010]

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Total Flow	:54.0 mL/min	
Column Flow	:1.00 mL/min	
Linear Velocity	:36.7 cm/sec	
Purge Flow	:3.0 mL/min	
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Splitter Hold	:OFF	
Oven Temp. Program		
Rate	Temperature(°C)	Hold Time(min)
-	70.0	0.00
8.00	260.0	2.00
4.00	280.0	5.00

[GC Program]

[GCMS-QP2010]

IonSourceTemp	:200.00 °C
Interface Temp.	:280.00 °C
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SECTION II

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GenBank ▾ Send to: ▾

Bacillus subtilis strain SFT1 16S ribosomal RNA gene, partial sequence

GenBank: OQ361800.1

FASTA Graphics

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  TITLE     Direct Submission
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Change region shown

Customize view

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Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Taxonomy

Recent activity

Turn Off Clear

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Chryseobacterium cucumeris strain PTEB2 16S ribosomal RNA gene, partial se — Nucleotide

Stenotrophomonas maltophilia strain QOS1

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GenBank ▾ Send to: ▾

Stenotrophomonas maltophilia strain TXM2 16S ribosomal RNA gene, partial sequence

GenBank: OQ361827.1

FASTA Graphics

Go to:

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ACCESSION   OQ361827
VERSION     OQ361827.1
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  ORGANISM  Stenotrophomonas maltophilia
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            Lysobacteraceae; Stenotrophomonas; Stenotrophomonas maltophilia
            group.
REFERENCE   1  (bases 1 to 672)
  AUTHORS   Johnson,J.V.K. and John,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-FEB-2023) Research and Post - Graduate Dept. of
            Zoology, St.Thomas' College, (Autonomous), Thrissur, Thrissur-
            Palghat Road, Thrissur 680001, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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FEATURES             Location/Qualifiers
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Change region shown

Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Taxonomy

Recent activity

Turn Off Clear

Stenotrophomonas maltophilia strain TXM2 16S ribosomal RNA gene, partial se(Nucleotide

Chryseobacterium cucumeris strain PTEB2 16S ribosomal RNA gene, partial se(Nucleotide

See more...

GenBank ▾ Send to: ▾

Pseudomonas aeruginosa strain FXE1 16S ribosomal RNA gene, partial sequence

GenBank: ON384042.1

FASTA Graphics

Go to:

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LOCUS       ON384042                 814 bp    DNA     linear   BCT 04-MAY-2022
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            Pseudomonadaceae; Pseudomonas.
REFERENCE   1  (bases 1 to 814)
  AUTHORS   K Johnson,J.V. and John,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-APR-2022) Research and Post - Graduate Dept. of
            Zoology, St.Thomas' College, (Autonomous), Thrissur, Thrissur-
            Palghat Road, Thrissur 680001, India
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Change region shown

Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Taxonomy

Recent activity

Turn Off Clear

Pseudomonas aeruginosa strain FXE1 16S ribosomal RNA gene, partial sequen Nucleotide

Stenotrophomonas sp. strain EON1 16S ribosomal RNA gene, partial sequen Nucleotide

Stenotrophomonas maltophilia strain DRNB1

GenBank ▾ Send to: ▾

Stenotrophomonas maltophilia strain DRNB1 16S ribosomal RNA gene, partial sequence

GenBank: ON384040.1

FASTA Graphics

Go to:

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            group.
REFERENCE   1  (bases 1 to 733)
  AUTHORS   K Johnson,J.V. and John,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (29-APR-2022) Research and Post - Graduate Dept. of
            Zoology, St.Thomas' College, (Autonomous), Thrissur, Thrissur-
            Palghat Road, Thrissur 680001, India
COMMENT     ##Assembly-Data-START##
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Change region shown

Customize view

Analyze this sequence

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Related information

Taxonomy

Recent activity

Turn Off Clear

Stenotrophomonas maltophilia strain DRNB1 16S ribosomal RNA gene, partial se Nucleotide

Paenibacillus alvei strain Q1T 16S ribosomal RNA gene, partial sequence Nucleotide

Paenibacillus alvei strain F1T 16S ribosomal

GenBank ▾ Send to: ▾

Paenibacillus alvei strain F1T 16S ribosomal RNA gene, partial sequence

GenBank: OQ361799.1

FASTA Graphics

Go to:

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REFERENCE   1  (bases 1 to 685)
  AUTHORS   Johnson,J.V.K. and John,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-FEB-2023) Research and Post - Graduate Dept. of
            Zoology, St.Thomas' College, (Autonomous), Thrissur, Thrissur-
            Palghat Road, Thrissur 680001, India
COMMENT     ##Assembly-Data-START##
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Change region shown

Customize view

Analyze this sequence

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Highlight Sequence Features

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Related information

Taxonomy

Recent activity

Turn Off Clear

Paenibacillus alvei strain F1T 16S ribosomal RNA gene, partial sequence Nucleotide

Bacillus subtilis strain SFT1 16S ribosomal RNA gene, partial sequence Nucleotide

Chryseobacterium cucumeris strain PTEB2

GenBank ▾ Send to: ▾

Chryseobacterium cucumeris strain PTEB2 16S ribosomal RNA gene, partial sequence

GenBank: OQ361849.1

FASTA Graphics

Go to:

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REFERENCE   1  (bases 1 to 626)
  AUTHORS   Johnson,J.V.K. and John,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-FEB-2023) Research and Post - Graduate Dept. of
            Zoology, St.Thomas' College, (Autonomous), Thrissur, Thrissur-
            Palghat Road, Thrissur 680001, India
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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Change region shown

Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Taxonomy

Recent activity

Turn Off Clear

Chryseobacterium cucumeris strain PTEB2 16S ribosomal RNA gene, partial se Nucleotide

Stenotrophomonas maltophilia strain QOS1 16S ribosomal RNA gene, partial se Nucleotide

Enterobacter cloacae strain XLD2P 16S

GenBank ▾ Send to: ▾

Paenibacillus alvei strain Q1T 16S ribosomal RNA gene, partial sequence

GenBank: OQ361777.1

FASTA Graphics

Go to:

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            Paenibacillus.
REFERENCE   1  (bases 1 to 532)
  AUTHORS   Johnson,J.V.K. and John,V.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-FEB-2023) Research and Post - Graduate Dept. of
            Zoology, St.Thomas' College, (Autonomous), Thrissur, Thrissur-
            Palghat Road, Thrissur 680001, India
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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Change region shown

Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Related information

Taxonomy

Recent activity

Turn Off Clear

Paenibacillus alvei strain Q1T 16S ribosomal RNA gene, partial sequence Nucleotide

Paenibacillus alvei strain F1T 16S ribosomal RNA gene, partial sequence Nucleotide

Bacillus subtilis strain SFT1 16S ribosomal

SECTION III

Name of antibiotics (dose)	Inhibitory zone diameter to nearest millimeter (mm)		
	Sensitive (S)	Moderately sensitive (MS)	Resistant (R)
Amoxicillin (30 μg/disk)	≥18	14–17	≤13
Cloxacillin (5 μg/disk)	≥25	22–24	≤21
Cephalothin (30 μg/disk)	≥18	15–17	≤14
Cephradine (25 μg/disk)	≥18	13–17	≤12
Cefuroxime (30 μg/disk)	≥23	15–22	≤14
Cefixime (5 μg/disk)	≥19	16–18	≤15
Kanamycin (30 μg/disk)	≥18	14–17	≤13
Streptomycin (10 μg/disk)	≥15	12–14	≤11
Neomycin (30 μg/disk)	≥17	13–16	≤12
Vancomycin (30 μg/disk)	≥12	10–11	≤9
Erythromycin (15 μg/disk)	≥23	14–22	≤13
Azithromycin (15 μg/disk)	≥18	14–17	≤13
Ciprofloxacin (15 μg/disk)	≥21	16–20	≤15
Levofloxacin (5 μg/disk)	≥17	14–16	≤13
Tetracycline (30 μg/disk)	≥15	12–14	≤11
Doxycycline (30 μg/disk)	≥14	11–13	≤10
Cotrimoxazole (25 μg/disk)	≥16	11–15	≤10
Chloramphenicol (30 μg/disk)	≥18	13–17	≤12

ANNEXURE

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International Journal of Zoological Investigations

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Biodegradation of Synthetic Pyrethroid Insecticide Deltamethrin by *Stenotrophomonas maltophilia* Strain DRNB1

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**Corresponding Author*

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Abstract: Deltamethrin is one of the most used pyrethroid insecticide in different regions of the world. It is the fast-acting insecticide that disrupts the cellular sodium channels. The deltamethrin residues are highly retained in environments, particularly in soil and water and have inevitable side effects on natural resources and human health. The most efficient, economical, and environmentally beneficial way of removing deltamethrin from contaminated sites is bioremediation. Bacteria are the most used biological agents in biodegradation studies. Thus, in this study deltamethrin-degrading bacteria *Stenotrophomonas maltophilia* strain DRNB1 was isolated and characterised. The bacterial growth was analysed by UV-spectrophotometer and deltamethrin degradation was studied by GC-MS analysis. *S. maltophilia* strain DRNB1 used deltamethrin as the sole carbon source for growth. Deltamethrin degradation efficiency of DRNB1 was 89.2% in insecticide supplemented media and 93% in presence of additional glucose in mineral media. These results implies that *S. maltophilia* strain DRNB1 could be used as a bioremediation technology for deltamethrin contaminated environments.

Keywords: Deltamethrin, *Stenotrophomonas maltophilia* DRNB1, Bioremediation, Soil, Pyrethroid

Citation: Josna Victoria K. Johnson and John Vimala K.: Biodegradation of synthetic pyrethroid insecticide deltamethrin by *Stenotrophomonas maltophilia* strain DRNB1. Intern. J. Zool. Invest. 8(2): 211-217, 2022.
https://doi.org/10.33745/ijzi.2022.v08i02.027

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Introduction

Pesticides are essential components of many agricultural management systems, and they can be used to control rodents, fungal infections, and weeds. Insecticides are used to reduce the spread of insect-borne diseases and agricultural pests in public health and agriculture, respectively (Damalas and Eleftherohorinos, 2011). The continued and widespread use of agrochemicals has negative impact on human health and causes several environmental consequences (Piutti *et al.*, 2002).

Deltamethrin - (s) – alpha – cyano – 3-phenoxy benzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclo propanecarboxylate) is a commonly used pyrethroid insecticide around the world with a broad spectrum of applications, primarily as an

acaricide and scabicide. They are synthetic substances designed to look like the pyrethrins found in chrysanthemum blooms. Because of its low cost, persistence, and durability, it has been widely used for more than 30 years all over the world. It is used to control pests such as mites, ants, weevils, and beetles on a variety of crops including cotton, corn, cereals, and vegetables since it is effective at low concentrations. Deltamethrin, which is particularly lipophilic, easily penetrates the cuticles of insects and acarines and kills them through ingestion. It functions as a neutrotoxin, causing the sodium channel activation gate to be inhibited. This interacts with y-aminobutyric acid receptors, causing neuronal transmission to be blocked (Ecobichon, 1991). Because of their phosphorylation, it also affects the action of chloride and calcium channels (Burr and Ray, 2004). It is identified in various environmental matrices as a result of persistent application, particularly in soil and water, where it can be hazardous to both target and non-target organisms (Hintzen *et al*., 2009).

The increased use of synthetic pyrethroids like deltamethrin have adverse health effects to human such as lymph node and splenic damage, carcinogens, and hormonal activity. Pyrethroids have cumulative neuroreproductive toxicity and endocrine disruption effects on nontarget animals (Hintzen *et al*., 2009; Kawahara *et al*., 2010). Chronic diseases may result from long-term exposure to certain insecticides (Osman and Abdulrahman, 2003). Some of them are considered to be carcinogenic (Pankaj *et al*., 2013). All of these elements combine to make pyrethroids potentially hazardous to human health and the environment. As a result, remediation measures to breakdown and eradicate pyrethroid residues from the environment are required. Bioremediation is considered as an environmentally and economically sustainable technology used for the removal of hazardous contaminants.

Soil microorganisms play a key part in this biodegradation process of deltamethrin (Chapman *et al*.,1981; Zhang *et al*.,1984; Grant and Betts, 2004). The aim of the present study was to isolate and characterize the deltamethrin degrading bacteria from tea garden soil.

Materials and Methods

Chemicals and media:

The pesticide used in the current study was reference standard deltamethrin with purity of (98%) obtained from Himedia. The chemical was dissolved in acetone for further studies. In the present study, degradation of the target compound (deltamethrin) only was studied, not its metabolites. All other chemicals were of analytical grade.

The MSM media for enrichment culture containing $2SO_4$ 2.0 g/l, KH_2PO_4 1.5 g/l, $CaCl_2.H_2O$ 0.01 g/l, $Na_2HPO_4.12H_2O$ 1.5 g/l was autoclaved (121 ^oC, 15min) and cooled. The media was supplemented with 50 mg/l of deltamethrin as the sole source of carbon and nitrogen is used. The pH of the medium was adjusted to 7.2 to evaluate biodegradation (Chen *et al*., 2011a, 2012).

Isolation of deltamethrin degrading bacteria:

The enrichment culture techniques were used for the isolation of deltamethrin degrading bacteria. For this purpose 20 g of tea garden soil of Annamalai hills region of Western Ghats (previously treated with deltamethrin) was added to flasks containing 100 ml of MSM supplemented with 50 mg/l of deltamethrin. Samples were incubated for 72 h on a rotary shaker (120 rpm) in a darkened thermostatic chamber maintained at 30±2°C after incubation, about 1 ml of soil suspension was transferred into flasks containing fresh MSM with the same concentration of deltamethrin and then incubated for an additional 72 h under the same condition. After seven subsequent transfers into the same medium, serial dilutions of the flask samples were plated onto MSM agar plates supplemented with 50 mg/l of deltamethrin to isolate the individual colonies (Cycon *et al*., 2014). Isolates exhibiting distinct

colony morphologies were isolated by repeated streaking on the same MSM agar medium.

Identification of bacterial isolates:

Isolates were characterised and identified using morphological features, biochemical tests and 16S rRNA gene analysis. The biochemical tests done were oxidase test, catalase tests, Lactose fermentation, motility test, gram staining, sulfide indole test and methyl red test.

Biodegradation experiment:

(A) *Inoculum preparation*:

Deltamethrin degrading strain designated as DRNB1 was used for the inoculum preparation. The bacterial strain was cultured in 100 ml Erlenmeyer flasks containing 20 ml of MSM supplemented with 50 mg/l of deltamethrin. The bacteria were pelleted by centrifugation (5 min, 10000 g) at the exponential phase. The pellet was washed twice with 0.85% of sterile NaCl and then resuspended in NaCl to obtain the bacterial suspension at a concentration of approximately 3 $x10^8$ cells/ml. The cell density (OD at 660 nm) was measured using a UV-VIS spectrophotometer.

(B) *Biodegradation of deltamethrin in MSM*:

The degradation studies were performed in 500 ml Erlenmeyer flasks containing 200 ml of sterile MSM supplemented with deltamethrin as the only source of carbon. The amount of insecticide applied is 50 mg/l. The medium was inoculated with 1 ml of bacterial suspension giving a final concentration of approximately $3x10^9$ cells/ml. Triplicate samples of MSM with strain DRNB1 as well as insecticide only used as controls. All samples were incubated on a rotary shaker (120 rpm) maintained at 30±1°C. Samples were removed periodically for bacterial growth rate and to determine deltamethrin concentrations. The growth of bacterial strains was recorded spectrophotometrically by measuring the OD at 660 nm using a UV-VIS spectrophotometer (Cycon *et al*., 2014).

(C) *Chemical analyses*:

To determine the deltamethrin concentration 10 ml of MSM was taken for analysis. Samples of MSM were filled to volume of 20 ml with deionised water and extracted twice with ethyl acetate. The extracts were dehydrated with anhydrous Na_2SO_4, evaporated to dryness under a stream of N_2 at 45°C using rotary evaporator and diluted to a final volume of 10 ml with hexane, and reserved for chromatographic analysis. Concentrations of deltamethrin were determined by gas chromatography. GC-MS column (Rxi 5SilMS), carrier gar helium and software GCMS solutions.

Results and Discussion

Isolation and characterization of deltamethrin degrading bacteria:

Four morphologically different bacterial isolates were obtained from tea garden soil through enrichment culture. The four isolates were selected to screen the deltamethrin degradation potential. The bacterial strain DRNB1, showed high tolerance to insecticide deltamethrin (50 mg/l) as a result, the strain DRNB1 was selected for further studies. The tea garden soil strain DRNB1 used deltamethrin as sole carbon source. The morphological, physiological and biochemical characterisation of bacterial strain DRNB1 was done. The characteristics were presented as follows: Gram negative rod shaped, non-motile, aerobic bacteria ,nitrate reduction test positive catalase positive, oxidase negative and mobile organism (Table 1).

Table 1: Morphological and Physiological characters of *S. maltophilia* strain DRNB1

Biochemical tests	Results
Shape	Rod shaped
Colony color	Bright yellow changes to dark brown
Gram staining	Negative
Motility	Non-motile
Catalase	+ve
Oxidase	+ve (slightly)
Nitrate reduction test	+ve

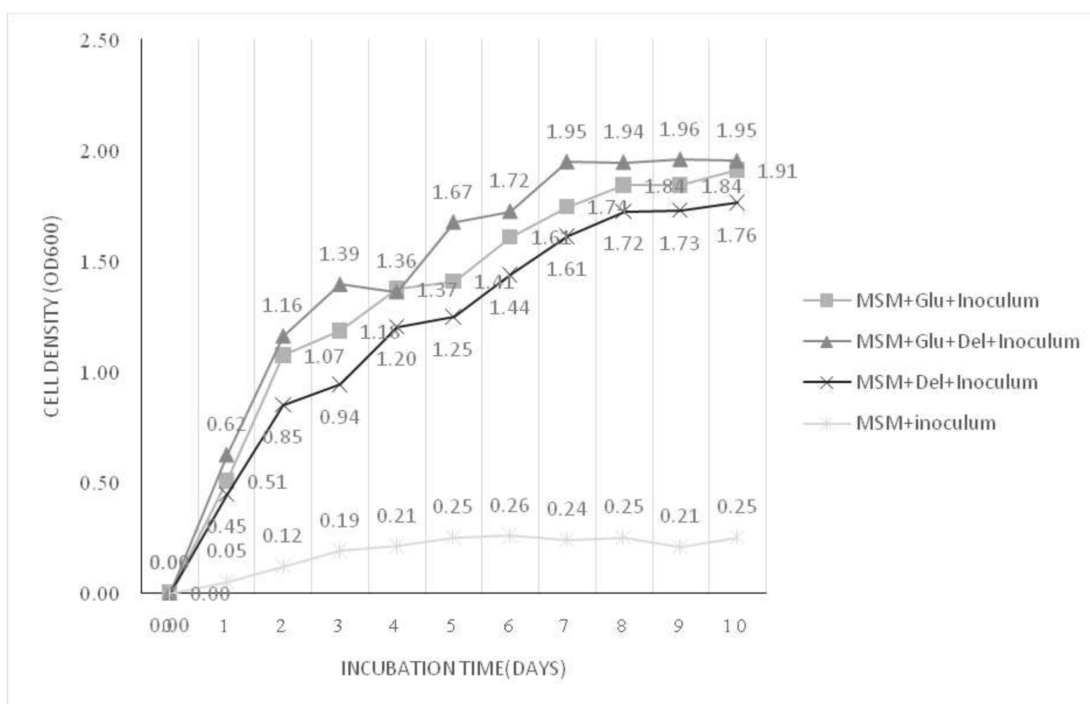

Fig. 1: Growth of bacterial strain DRNB1 in different Mineral Salt Media. MSM-Mineral Salt Media, Glu-Glucose, Del- Deltamethrin and Inoculum- DRNB1.

The 16S rRNA partial gene amplification of strain DRNB1 (733 bp) and BLAST search showed the similarity between DRNB1 and *Stenotrophomonas* sp. (accession number – CP052863.1). Therefore, this tea garden soil strain was named as *Stenotrophomonas maltophilia* DRNB1 and deposited in GenBank under the accession number ON384040.

Degradation of deltamethrin by S. maltophilia DRNB1 in MSM:

The growth of bacteria is also increased in the presence of glucose. Maximum bacterial growth, cell density 1.94 was obtained on the 8th day (Fig. 1). The degradation rate of deltamethrin in the control flasks (MSM and deltamethrin, without the strain DRNB1) was about 15% after 10 days of incubation (Fig. 2).

The addition of glucose increased the growth of bacteria and biodegradation of deltamethrin. In the absence of glucose, the deltamethrin degradation efficiency of *S. maltophilia* DRNB1 was 89% at the end of 10 days. The addition of 1 g/l of glucose increased the deltamethrin degradation efficiency of *S. maltophilia* to 92%, which corresponds to an increase of 2.8% (Fig. 2).

The *Stenotrophomonas* species has been shown to degrade the variety of hazardous compounds like polycyclic aromatic hydrocarbons (Juhasz *et al*., 2000), acrylamide (Lakshmikandan *et al*., 2014), acetamiprid (Tang *et al*., 2012), endosulfan (Barragain-Huerta *et al*., 2007; Kumar *et al*., 2007) a wide range of pyrethroids such as fenvalerate, deltamethrin, β-cypermethrin and cyhalothrin (Chen *et al*., 2011b), 4-substituted phenols (Liu *et al*., 2009), herbicide butachlor (Dwivedi *et al*., 2010), diuron (Batisson *et al*., 2007; Egea *et al*., 2017; Silambarasan *et al*., 2020), α-endosulfan (Ozdal *et al*., 2017) and diazinon (Pourbabaee *et al*., 2018). Gur *et al*. (2014) reported that *S. maltophilia* OG2 could degrade 69.9% of 100 mg/l cypermethrin after 10 days incubation. Wu *et al*. (2021) reported that *S. maltophilia* XQ08 could degrade 63.26% of 100 mg/l deltamethrin after 5 days incubation. An inoculation of soil contaminated with fenvalerate (50 mg/kg of soil) with *Stenotrophomonas* sp.

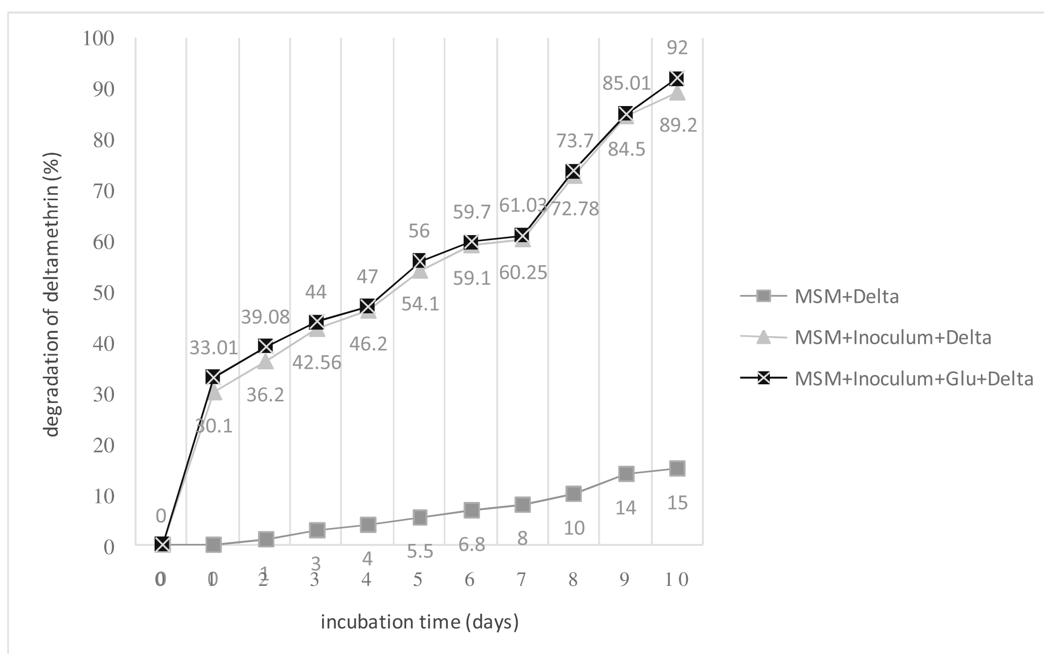

Fig. 2: Degradation of deltamethrin in Mineral Salt Media supplemented with glucose and deltamethrin. MSM with deltamethrin without DRNB1 is taken as control.

Strain ZS-S-01 increased the rate of pyrethroid dissipation, and its half life value was eight times lower than for soil without strain (Chen *et al*., 2011b).

Bacteria capable of degrading deltamethrin were mostly isolated from agricultural areas where intensive pesticides were used. Chen *et al*. (2012) found that *Streptomyces aurus* HPS-01 could degrade cypermethrin. Song *et al*. (2015) studied the deltamethrin biodegradation with *Pseudomonas aeruginosa* JO-41 strain isolated from the pyrethroid contaminated soil. *Acinetobacter calcoaceticus* MCm5, *Brevibacillus parabrevis* FCm9, *Sphingomonas* sp. Rcm6, *Bacillus megaterium* JCm2, *Ochrobactrum anthropic* JCm1 and *Rhodococcus* sp. JCm5 were used in biodegradation of deltamethrin (Akbar *et al*., 2015a, b). Cycon *et al*. (2014) isolated deltamethrin degrading *Serratia marcescens* Del-1, Del-2 from insecticide treated soil. The other deltamethrin degrading species are *Bacillus cereus* Y1 (Zhang *et al*., 2016), *Lysinibacillus fusiforms* ZJ6 (Hao *et al*., 2018), *Acinetobacter baumannii* ZH-14 (Zhan *et al*., 2018). Kumral *et al*. (2020) reported deltamethrin degrading *Lactobacillus plantarum*, which is used as a food fermenter. The efficiency of pesticide degradation increased with the addition of extra carbon sources (Kumar and Philip, 2006; Chen *et al*., 2011a, 2012).

Conclusion

In this study, a deltamethrin degrading bacterial isolate *Stenotrophomonas maltophilia* DRNB1, was isolated from tea garden soil by enrichment technique. Strain DRNB1 utilizes deltamethrin as a sole source of carbon. Environmental problems due to pesticide contamination is one of the major problems. Biodegradation of the pesticides is the efficient method to remediate pollutants from the contaminated sites. Therefore, this study showed that *Stenotrophomonas maltophilia* DRNB1, can be used as a bioremediation tool to eliminate contaminants from polluted sites.

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