STUDIES ON *NECROBIA RUFIPES* (COLEOPTERA, CLERIDAE), A PEST OF DRIED FISH AND ITS CONTROL

By

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DECLARATION

I, Abdul Rabbi Nistar, C.K., hereby declare that this thesis entitled **"Studies on *Necrobia rufipes* (Coleoptera, Cleridae), a Pest of Dried fish and its Control"** submitted to the University of Calicut in partial fulfillment of the requirements for the Doctoral degree in Zoology, is a bonafide research work done by me under the supervision and guidance of Dr. Nasser, Senior Lecturer, Department of Zoology, University of Calicut.

I further declare that the thesis has not previously formed the basis for the award of any other degree, diploma or similar title.

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C E R T I F I C A T E

This is to certify that this thesis entitled **"Studies on *Necrobia rufipes* (Coleoptera: Cleridae), a Pest of Dried fish and its Control"** is an authentic record of the bonafide research work carried out by Mr. Abdul Rabbi Nistar, C.K., under my supervision and guidance and that no part of this work has been presented before for any other degree or diploma. It is further certified that Mr. Abdul Rabbi Nistar, C.K., has completed his M.Phil. from the University of Calicut.

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To My Dear Brother,

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CONTENTS

INTRODUCTION		1-6
CHAPTER I	**REVIEW OF LITERATURE**	7-34
	1) Distribution	7-8
	2) Biology	8-11
	3) Temperature and Humidity	11-18
	4) Mating Behaviour	18
	5) Deterrents and Antifeedants	18-34
CHAPTER II	**MATERIALS AND TECHNIQUES OF STUDY**	35-40
	1) Maintenance of Stock Culture	35
	2) Biology	35
	3) Fecundity	35-36
	4) Longevity	36
	5) Courtship and Mating Behaviour	36
	6) Preparation of Plant Extracts	36-38
	7) Spices oils	37
	8) Survey	38-39
	9) Statistical Analysis	39-40
CHAPTER III	**DEVELOPMENTAL BIOLOGY**	
Effect of different Temperature and Photoperiodicity on Biology		
	i) Results	41-47
	ii) Discussion	47-52

Influence of Incubation Period and larval instars on subsequent instars

i) Results 53-62

ii) Discussion 62-65

CHAPTER IV LONGEVITY

Effect of Temperature and Photoperiodicity on Longevity

i) Results 65-66

ii) Discussion 66-68

CHAPTER V REPRODUCTION

Courtship and Mating Behaviour

i) Results 69-70

ii) Discussion 70

Effect of Temperature and Photoperiodicity on Fecundity

i) Results 71-75

ii) Discussion 75-82

CHAPTER VI FOOD CONSUMPTION

Food consumption at different Temperature and Photoperiod

i) Results 83-84

ii) Discussion 84-85

CHAPTER VII EFFECT OF LEAF EXTRACTS AND SPICE OILS

i) Results 86-88

ii) Discussion 88-93

CHAPTER VIII FIELD SURVEY 94-113

CHAPTER IX SUMMARY 114-117

CHAPTER X REFERENCES 118-139

INTRODUCTION

INTRODUCTION

Insect infestation of traditionally cured fish by flies and hide beetles is still an important cause of post- harvest losses in many developing countries. Studies on causes and means of control have been continuing for the past many decades. Insects of Order Coleoptera and Diptera are often found infesting cured fish during and after processing especially in tropics and subtropics. The problem exists in the curing site where insects are found to infest cured products during drying and on storage tropics. Considerable weight loss of the product occurs. The product quality is lost and has to be degraded as manure. Financial losses occur to the fisherman whose sole means of sustenance during off-season are the returns from curing activities. Cured fish can also suffer considerable loss of weight due to feeding by insect. Fish is susceptible to beetle infestation once the moisture content is lowered. Thus, fish is subject to beetle infestation throughout storage and transportation, and so the potential for losses is great. Under adverse condition quantitative losses of up to 90% due to fly damage during processing and losses ranging from 20-50% caused by the fish beetle notably, *D. maculatus* and *N. rufipes* have been reported on cured fish. These pests proliferate and grow on the dried fish, thus changing its appearance and powdering the fish making it unfit for marketing. In addition to this, insect pests of fish often transmit *E coli*, mould spores and heat and moisture produced by heavy infestation can create conditions suitable for mould growth on fish that has previously been dried.

The major pests are blow flies (Diptera: Calliphoridae and Sarcophagidae) and hide beetles (Coleoptera: Dermestidae and Cleridae). The extent of losses incurred varies considerably between locations, and can even fluctuate at a single site. The main beetle pests of cured fish are *Dermestes*

species and *Necrobia rufipes. N. rufipes,* commonly known as red – legged ham beetle belongs to the family Cleridae and is commonly found on cured fish in many regions of the world (Riley, 1874). The insect is also known by the name “paper worm” and “copra bug”.

The adult insects are extremely mobile and females lay their eggs on the fish; these hatch and the larvae eat their way in to the dried flesh. In Kerala as a whole these insects cause storage losses; some of the losses are due simply to loss of weight, but in some cases the fish becomes unfit for sale.

Although considerable amount of studies have been carried out to develop appropriate insect control measures for these pests on cured fish, a systematic effort to effectively manage the insect is lacking.

Initial infestation is normally through adults locating the fish, and then depositing their eggs upon it. Both the adults and the larvae feed upon the fish, causing large quantitative losses of edible material, and fragmentation of the remaining product. Beetle infestation is of sufficient magnitude to require the application of loss reduction strategies.

The important control measures may be categorized as either physical or chemical methods. Physical methods involve the use of some kind of physical barrier, such as a screen or a sealed box, to prevent the insects from gaining access to the fish. Chemical methods involve the use of insecticides or fumigants to destroy the insects, or chemical deterrents, such as salt or plant extracts, which repel the insects from fish. These methods have not met with sufficient success, as the causative factors of pest infestation have not been tackled.

The likelihood of infestation occurring, and its subsequent severity, is thought to be influenced by the interaction of many parameters. Some of these

parameters, such as climate, season, and site locality, are largely beyond the control of processor. Large number of poor people in many tropical countries depends upon fish processing for their livelihood and is a low investment business (Johnson & Esser, 2000)

For the poor fishing communities, fish processing offers an opportunity for income generation that requires relatively low investment. Being a traditional household-level activity, it is relatively easy for poor people to acquire the skills and social approval necessary to take up fish processing. The socio- economic conditions in fishing villages allow processors to enter into a range of relationships with producers, other members of the community and buyers (traders and consumers), facilitating flexibility of operation, financial security and occupational sustainability. The socio-cultural milieu in which traditional fish processing is conducted help processors to access, virtually free of cost, many services that would otherwise make processing less cost efficient, or even unprofitable. Traditional fish processing is thus an outcome of a sensitive balance and integration between different technical, socio-economic, cultural and environmental factors (Esser *et al*., 2003)

In many fish drying centres all over Kerala, small scale fish drying for human consumption is done mostly by women on a small scale. Esser *et al.*, (2003) has also noted that a large majority of people involved in traditional fish processing are women e.g. on the east coast of India, more than 90% of fish processing is done by women. In rural areas, the fisher's traditional role in the production cycle ended when he landed his fish catch on the shore and handed it to his wife or other female members in his family to process. The women, in turn, carried the fish to the processing areas near their houses, and processed them into salted and dried products before selling them in the market and using the income to manage the household. Before ice and transport systems became widely available, traditional fish processing was the

main channel through which a household earned its income, and women were the main earners in the household. Even now, despite more fish entering the cold chain, this pattern continues to exist in certain parts of India and elsewhere, particularly in remote villages.

Traditionally processed fish includes fish that are salt-dried, wet-salted, dried without salting, and smoked. Despite a general decline in traditional fish processing over recent years, there remains a strong market demand for traditional fish products, which continues to ensure livelihood and food security for a substantial number of poor dried fish producers and consumers.

With fish supplies becoming increasingly scarce, due to depletion of fish catches and increased competition from fresh fish traders, the need for traditional processors to reduce losses and increase value-added is becoming critical.

Research has demonstrated that by adopting a systematic and participative approach to introducing simple infestation control measures, processors can significantly reduce losses and improve product quality, there by raising the incomes of processors and increasing food security for poor consumers, many of whom depend on dried fish as their main source of animal protein (Esser *et al*., 2003).

One solution to these problems might be to replace synthetic chemicals with compounds, which occur naturally in plants. Studies carried out by Okorie et al., 1990; Mathen et al., 1992; Golob and Webley, 1980; Wood 1982; Don Pedro 1996a;1996b; Walker and Wood 1986) have shown that plant extracts show much promise as protectants of dried fish.

In much of the developing world fish is preserved by curing, Sun-drying with or without salt and by smoking. Many artisanal fishing

communities do not have access to ice or cannot afford it, so curing is the only means of preserving fish for distribution and marketing.

To protect their fish from insect infestation some processors and traders use insecticides, which are applied as sprays, dips, or in powder form. Insecticide residues are potentially harmful to humans as they can persist in the fish during processing and cooking.

There is awareness on a governmental level of the dangers of using insecticides on food. Many countries have banned the use of insecticides on cured fish. Unfortunately, in many instances such formal bans are not effectively imposed and insecticide use continues.

All chemicals pollute the environment and are hazardous to use. Furthermore, insects are becoming increasingly resistant to insecticides. These reasons have led to a general reluctance to use or to encourage the use of any insecticides, even those approved by the JMPR. Consequently alternative methods for controlling insect infestation of fish are needed, and various methods have already been investigated, including salt (Osuji 1975), proper hygiene practices, smoking, solar driers, packaging (Proctor 1977); irradiation (Ahmed et al. 1978), screening (Esser et al. 1990; Esser 1991). However, for socioeconomic, technical or marketing reasons it is impractical to rely only on one of the methods for adequate control of infestation.

The earliest recorded attempts at pest control were mainly concerned with the biology of the pests and their ecology, and attempts were made to make the environment less favourable for the pests by cultural and physical means.

The use of lethal temperatures, both high and low, for insect pest destruction is of importance in many countries. The purpose of this method is not the actual destruction of the pests but the drastic retardation of

development following the reduction of the metabolic rate. The drying of fish, which is widely practiced for a reduction in moisture content, usually results in lower infestation rates by most pests.

In order to devise control measures for any insect pest it is essential to study its developmental biology and reproductive behaviour and how they are influenced by temperature, photoperiod and humidity. The present study is a step forward to fill in the lacunae in our knowledge about *N. rufipes* and hence to plan a systematic approach for its control. Preliminary studies on the role of plant extracts and spice oils on development have also been carried out.

CHAPTER I

REVIEW OF LITERATURE

REVIEW OF LITERATURE

DISTRIBUTION

Roesli and Subramanyam, (2002) noted the red-legged ham beetle (RLHB; *Necrobia rufipes*), a pest of stored products such as copra, ham, cheese, dried fish, and other protein-rich foods. *N. rufipes* infests various commercially important stored commodities in Nigeria and the development of *N. rufipes* on several commodities (dried fish, copra, cacao beans, palm kernels, groundnuts, and maize) were studied (Osuji, 1977). Development was completed in dried fish (where it was most rapid), palm kernels, groundnuts, and copra but not in cacao beans or maize. Occurrence of *N. rufipes* damaging cashew nut was recorded by Pratissoli (1997). In a survey of godowns in Kerala, India, *N. rufipes* and *O. surinamensis* were found to be the most important pests (Kumari *et al*., 1992). Investigations on insect pests of stored oil-palm kernels (Allotey and Kumar, 1989) established the value of *N. rufipes* as a predator. *N. rufipes* was also found in a museum infesting the crevices and spongy parts of the bones of a recently prepared skeleton of a whale at Kozhikode, Kerala. (Adolph and Soans, 1969).

A survey in the market in Ibadan, Nigeria in the period from January 1971 to July 1972 showed that Coleoptera, especially *Dermestes maculatus* (which accounted for 71.5% of the observed infestation) and *N. rufipes* (28.0%), infested a high proportion of the dried fish sold. Although both species were abundant throughout the year, infestation was highest in the hot dry months and lowest in the rainy ones. *Tribolium castaneum* and *Trogoderma granarium* were found in considerable numbers. (Osuji, 1974 a).

The insect fauna on stored palm produce was studied in Nigeria in a transit shed (Allotey, 1988). The pooter search method revealed individuals of the clerid *N. rufipes* throughout the year, despite fumigation with phostoxin (aluminium phosphide).

When samples of dried fish of the genera viz. *Citharinus*, *Clarias*, *Heterotis* and *Synodontis* on sale in the market in Ibadan, were examined the numbers of examples of *D. maculatus* and *N. rufipes* found per 100 g fish averaged 6.4 (1.53), 59.5 (24.5), 33.7 (19.2) and 29.5 (9.65), respectively . The lipid contents of the samples averaged 12.29, 16.64, 12.87, and 13.42%, for the four genera respectively (Osuji, 1974 b).

BIOLOGY

Preliminary observations on the biology of the *N. rufipes* were carried out by Simmons and Ellington (1925). They made some observations on the biology and behaviour of *N. rufipes* and gave general accounts on the eggs, hatching, larvae, pupae and adults and also discussed some life history information such as oviposition, incubation period, fecundity, developmental period, pupal period etc. The fecundity was an average of 137 eggs and the incubation period was reported to be 4-5 days.

The development of *D. maculatus* in dried fish was studied under uncontrolled laboratory conditions in Nigeria (Osuji, 1975d). Females laid eggs within 12 h of copulation, and oviposition was improved by the presence of free water. Hatching occurred about 48 h after oviposition. Larval development was completed in 33.5 days, during which seven moults occurred and a body length of 14 mm was attained. It was also observed that crowding prolonged larval life. When intact pieces of fish were available, the last-instar larva bored into one of them and pupated within the hardened larval skin, but when ground fish was provided, a quiescent pre-pupal stage was

observed. The adult emerged about 11 days after the end of the last larval instar, irrespective of the mode of pupation.

Investigations were made on the biology of *Typhaea stercorea, N. rufipes, Attagenus simulans* and *A. augustatus*. *Typhaea stercorea,* of which the larvae feed on the germ and the adults on the endosperm of the grain, had two generations a year and overwintered as an adult. *N. rufipes* had one generation per year, the adults likewise overwintering. Both larvae and adults fed on the germ of grain with moisture content of 12% and over. The larvae of *A. simulans* and *A. augustatus*, but not the adults, were injurious to grain (Ya, 1970).

Studies on varietal preference, growth and development of pests *N. rufipes* and *Orizaephilus surinamensis* in stored copra (Kumari and Mamman, 1998) revealed that the severity of infestation varied with varieties and the growth index varied with respect to larval period, larval mortality, pupal period, pupal mortality, adult emergence, adult longevity, and fecundity.

A comparative assessment of the biological performance of *D. maculatus* in various dietary media namely dried fish, fish meal, bone meal, palm kernel meal, blood meal, and whole meal has been made (Osuji, 1978) and the dried fish followed by fish meal was found to be significantly superior to the commercial feeds. He suggested that the greater suitability of the dried fish diet for the development and biological performance of the beetle might be attributable to its superior nutritional composition in respect of crude proteins, total lipids, and water content, among other factors.

Elbert (1978) studied the biology of *Trogoderma variabile* including the development and diapause of larvae. The results indicated that development of *T. variabile* in unheated room is possible. Because of the very

slow development at lower temperatures economically important damages are unlikely.

Some aspects of the life patterns of *D. maculatus* and *Dermestes lardarius*, had been described by Schmidt (1974). He observed that the beetles and larvae consumed mainly pure fats or food substances with fatty components. The hair like sense organs in the antennae was used for the reception of smell.

Bhuiyan and Saifullah (1997) studied some aspects of the biology of *N. rufipes* using a mixture of dried fish and copra as food. The average longevity of adults was found to be 60.64 ±39.46 days for females and 49.42 ±18.2 days for males. The mean fecundity recorded was 89.7±17.8 eggs with arrange of 0-350 eggs per female. The hatchability was 89.59±7.27 percentages. Egg lying continued until the death of the female.

Azab *et al*., (1973 a) studied the biology of *D. maculatus* in Egypt. When reared in the laboratory on dried meat, 5-6 overlapping generations developed in a year. Both males and females paired several times, and the females laid their eggs singly or in groups of up to 25. Adult males and females lived for up to 189 and 178 days, respectively in autumn (21.5^{o}C). The sex ratio was 1:1.

Nath and Pande (1996) examined the biology of *D. maculatus* under uncontrolled laboratory conditions on dried fish in north –east India. Pre-oviposition, oviposition and post-oviposition periods lasted for 5 to 6 days , 40 to 47 days, and 8 to 10 days, respectively. The fecundity varied from 29 to 95 and spectacularly improved by the addition of wet cotton. The egg stage lasted for 36 to 48 hours. Larvae underwent 7 instars and the development was completed in 25 to 30 days. Larval duration was prolonged by crowding.

Pupation took place in the crevices of the fish body and lasted for 5 to 7days. Adults were long-lived and polygamous.

Cordingley (1980) discussed the general biology of *Phalaenoides glycine* including rates of development at different temperatures. The optimum temperature range for larval development was 15-27°C and threshold 10.1°C approximately.

EFFECT OF TEMPERATURE AND HUMIDITY ON DEVELOPMENT AND FECUNDITY

Toye (1970) studied the humidity and temperature reactions of adult *D. maculatus* Deg. with reference to infestation on dried fish and was found to have stronger preference than larvae for the higher humidity.

In an investigation on the development, fecundity and longevity of *Demestes ater* at various combinations of temperature and humidity, the last larvae and adults were found to avoid a temperature range of 30-45ºC. Longevity recorded a maximum of 100 days at 25ºC and the fecundity was very variable (0-135) as was the percentage hatch (0-69 %) (Coombs, 1981).

Both *Dermestes haemorrhoidalis* and *D. peruvinus* (Coombs, 1979) developed at all humidities tested at 25ºC and the rate of oviposition found to be diminishing with age of female.

Howe (1953) observed that relative humidity has a marked effect than temperature on the development of *Dermestes frischii*. Pupal period was shortest at 37ºC.

Life cycle of *Dermestes lardarius* was completed in 223 days at 17.5 and 20ºC, but at 30ºC it took only 55 days. Temperature had a great influence on the number of eggs laid. The total yield of eggs reached a peak of 1261 at 20ºC and then declined to 52 at 27.5. No eggs were laid at 30ºC. Optimal

temperature for egg laying in the species was found to be 20ºC (Jacob and Fleming, 1984 a).

An investigation in to the effect of illumination intensity on the response of the hide beetle, *D. maculatus*, to aggregation pheromone (Rakowski, 1988) revealed that the intensity of light do play an important role in the behaviour of *D. maculatus*.

Larval and pupal development at different moisture levels and on various media were examined (Scoggin and Tauber, 1951) and the results established that when the water content was maintained between 10-15 percentages, larval mortality was lowered and the number of larval instars and duration of larval development decreased, and larger adults emerged.

An inquiry was made in to the effect of temperature and relative humidity upon the development and fecundity of *D. lardarius* (Coombs, 1978) suggested that development of the female *D. lardarius* took longer than that of the males. Shortening of larval period was observed at high humidities. Development was more rapid at higher temperature Unsuccessful pupation was the rule at 15ºC and at 30ºC none of the adult pairs laid eggs. The fertility of the eggs was very variable and only of the order of 50 percentages.

The duration and viability of the egg stage of *D. maculatus* were determined using a wide range of constant temperatures and humidities (Jacob and Fleming, 1985) Temperatures over which the eggs hatched greatly influenced the duration of the egg stage but had little effect on viability, except at the lowest temperatures studied (15 and 17.5°C). The mean duration of the egg stage varied from about 2 days at 37.5 °C to up to 17 days at 15°C. Humidity had little effect on the incubation period except at the lowest

temperatures, but had a marked effect on viability. Viability was highest at 90% R.H and lowest at 20% R.H.

The duration of development, pattern of egg lying, and fecundity of *Dermestes haemorrhoidalis* were investigated on a fishmeal diet at various temperatures and relative humidities (Jacob and Fleming, 1984 b). At 65% R.H., eggs failed to hatch at temperatures below 15 or above 32.5°C; the mean duration of the egg stage varied from 2.6 days at 32.5°C to 11.6 days at 15°C. At 25°C, neither the duration of the egg nor egg viability appeared to be affected by humidities in the range 20-90%. At 65% R.H., there were 6-8 larval instars at 20 and 25°C and 7-9 at 30°C. Humidity also affected the number of instars. The shortest mean larval developmental period was 37.3 days at 30°C, 65% RH., and the longest was 76.0 days at 20°C, 65% R.H. Adults were comparatively long-lived and laid higher numbers of eggs for longer periods at 20°C than at other temperatures. Below 20°C, fewer eggs were laid, and above this temperature adult lifespan and fecundity declined.

The life-span and fecundity of mated pairs of adults of *Dermestes lardarius* were compared at 25°C and 65% R.H. when water was not provided, given once a week, once a day, or supplied continuously. It was found that the moisture content of the food alone did not support adult life for long. Drinking water was required at least once a week to achieve the maximum life span, and was needed more often if females were to be more productive. The longest oviposition periods were obtained when water was provided most often (Jacob and Fleming, 1982).

Thornton (1981) evaluated the effect of temperature on the growth and development of a South African strain of *D. maculatus*. At constant temperatures of 20-35°C, the incubation period averaged 6.7-2.1 days, and the larval, pre-pupal and pupal periods averaged 43.9-20.8, over 10 to 4, and 11.1-6.0 days, respectively. No pupae formed at 20°C. Females kept at 35°C

laid an average of 605 eggs, as compared with 1129-1464 at lower temperatures. Egg mass was inversely related to temperature, averaging 0.33 mg at 20°C and 0.18 mg at 35°C.

Adult females of *D. lardarius* maintained at 27.5°C and 80% R.H. began to lay eggs when 10-21 days old, continued laying for 7-21 days, and laid up to 17 eggs, of which less than 50% hatched. At 20°C and 80% R.H., most females began to lay when 57-101 days old, and produced 14-58 eggs in 14-119 days, about 50% of them fertile. At 25°C and 80% R.H., most females laid some eggs when a few days old; there was then a gap of 14 weeks or more before the rest of the eggs were laid. Lower humidity (65% R.H.) appeared to eliminate early oviposition (Jacob and Fleming, 1981).

Fleming and Jacob (1986) studied the influence of temperature and relative humidity upon the number and duration of larval instars in *D. lardarius*. Under most conditions and, contrary to the findings of other workers, number of instars varied. At 65% R.H., the number of instars increased from the usual 6 at 20°C to 8 at 27.5°C. At 25°C, the number of instars decreased from 8 at 50% R.H. to 6 (for most larvae) at 80% R.H. At 65% R.H., the shortest mean larval development period was 51.5 days (at 27.5°C) and the longest was 109.7 days (at 17.5°C). Mortality was high at 17.5 and above 22.5°C. It was concluded that the optimal conditions for development were 20-22.5°C and 65% R.H.

Larvae of *D. lardarius* were bred at 80% R.H. and 15 or 30°C on a diet of fishmeal, yeast and cholesterol. Adults obtained at 15°C were morphologically normal and lived up to 211 days; most females failed to lay eggs but 2 laid a small number (4 and 12) of infertile eggs after 89 days. At 30°C, adults lived only up to 36 days and none of the females laid eggs. Larvae were also reared at 65% R.H. and 15 or 30°C until they pupated and then they were transferred to 20 and 25°C, respectively. Adults at 25°C lived

up to 61 days but laid no eggs. Adults obtained at 20°C were morphologically normal but very small in size and lived up to 165 days; mating between them was infertile (Jacob and Fleming, 1980 a).

Duration of the egg stage of *D. lardarius* and percentage hatch at various combinations of temperature and relative humidity were examined (Jacob and Fleming, 1980 b). As the temperature increased from 15 to 32.5°C, the duration decreased from 12.9 to 3.0 days and percentage hatch from 50.8 to 12.5. Relative humidity had little effect on either duration or percentage hatch. When eggs were kept only at 25 and 30 °C and 65 and 90% R.H., percentage hatch ranged from 34 to 44.

Study on the biology of the beetle, *D. maculatus* was conducted (Pisfil and Korytkowski, 1974) at 24°C and 70% R.H, fishmeal in the form of powder or pellets being provided as food. The egg stage lasted for an average of 3.4 days, the six larval instars together averaged 21.05 days, the pre-pupal and pupal stages averaged 4.8 and 8.3 days, respectively, and the adult males and females lived for averages of 116.15 and 114.35 days, respectively.

A study was conducted to determine the effects of temperature and humidity on the development of stored products pest, *D. maculatus* (Majeed, 2002). Treatments comprised: 28±2, 32±2 and 38±2°C with 40±5 and 60±5% RH at each level of temperature. Development of adults was more adversely affected by temperature compared to relative humidity. The longest duration of maturation (6.70±0.36 days) was obtained at 28±2°C and 60±5% RH. The shortest duration 4.90±0.97, 23.1±0.72 and 3.70±0.31 days for maturation, oviposition and post-oviposition, respectively, was recorded at 38±2°C and 40±5% RH. An increase in temperature at constant relative humidity affected the development and decreased the maturation, oviposition, post-oviposition and longevity of adults. An increase in relative humidity decreased the rate of maturation, oviposition, post-oviposition and longevity at a particular

temperature. The larval survival percentage decreased with temperature increase. Temperature and relative humidity affected the larval duration and developmental index. However, the larvae developed faster at higher temperatures and lower humidities. Temperature and humidity affected the pupal period and survival. The pupae developed faster at higher temperature and lower humidity.

Azab *et al*., (1973 b) investigated the effects of temperature, relative humidity and type of food on the duration of the immature stages of *D. maculatus*. At 75% R.H. and 21, 27 or 35^{o}C., the egg stage averaged 5.91, 3.02 and 1.87 days, respectively. At 55^{o}C R.H., the larval stage averaged between 18 days at 35^{o}Cand 64 days at 21^{o}C; at 27^{o}C; it averaged 22 days at 75% R.H. and 46 days at 55% R.H., the pupal stage averaged between 4 days at 35^{o}C and 13 days at 21^{o}C; relative humidity appeared to have little effect on the duration of this stage. At 75% R.H.., the adults lived for a mean of 169-173 days at 21^{o}C and 49.1-51.9 at 35^{o}C. Adults of both sexes lived longer at 55% than at 75% R.H.

Survey on factors influencing the rate of oviposition in *D. maculatus* (Azab *et al*., 1973c) revealed that when the relative humidity was 75%, females kept at 21, 27 or 35^{o}C laid average totals of about 214, 362 and 83 eggs each, respectively, and the oviposition period averaged about 130, 92 and 28 days, respectively. At 27^{o}C and 55% R.H., the average total number of eggs laid/female was about 333 and the oviposition period averaged about 86 days. Females oviposited normally only when they had been provided with water in addition to suitable food. To obtain maximum numbers of eggs it was necessary to keep males and females together continuously.

The reactions to temperature of the larvae of *D. maculatus* were studied using a radial temperature gradient apparatus (Osuji, 1975 c). The larvae consistently avoided the hottest zones of the gradients (39.5 or 44^{o}C)

and were preferentially distributed in the cooler zones (23.5-25^{o}C or 32 - 34^{o}C).

Nakahira and Arakawa (2005) studied the effect of photoperiod on development of the green lacewing, *Chrysopa pallens* and found that the larval developmental period was affected by photoperiod.

Whittaker and Kirk (2004) investigated the effect of photoperiod on the behaviour of adult females of the western flower thrips, *Franklinella occidentalis* (Pergande) (Thysanoptera: Thripidae) at 25°C. The amount of walking, pollen consumption, and oviposition all increased with increasing photophase.

Nakamura (2003) examined the effect of photoperiod on *Dolycoris baccarum* nymphal development; growth and adult size and found that the developmental period was longer under short rather than a long day photoperiod. Adult size was largest under an intermediate photoperiod of L13: D11, and was smaller under both longer and shorter photoperiods.

Ekesi *et al*., (1999) studied the effects of temperature and photoperiod on development and oviposition of the legume flower thrips, *Megalurothrips sjostedti* and at constant temperatures, the highest pre-oviposition period was observed at 29°C under a photoperiod of L16:D8. Egg production also ceased at this temperature/photoperiod combination.

At 23-25^{o}C and 80-90 % RH, the oviposition period of *D. maculatus* lasted for up to 73 days and female laid up to 270 eggs each; the larval stage averaged 40 days and the pupal stage 12 days, and the adult life-span 76 days for females and 70 days for males (Shahhosseini, 1980).

Amos and Morley (1971) investigated the longevity of *Dermestes frischii* at two temperatures (30 and 35^{o}C), four relative humidities (30, 45, 60

and 75%) and three salt contents (14, 25 and 60%). At 30^{o}C, the average duration of adult life varied from about 12 days (at the lowest R.H. and high salt content) to nearly 60 days (at high R.H. and no salt content). At 35^{o}C, the effects of humidity and salt content were similar but less marked, and adult life lasted about 12-25 days.

MATING BEHAVIOUR

In the oriental beetle, mating and copulation occurred without an obvious complex courtship, but observations of post mating behaviours suggested that mate guarding occurs (Facundo *et al*., 1999).

Edvardsson and Arnqvist (2005) examined the effect of copulatory courtship on differential allocation in the red flour beetle, *Tribolium castaneum*, which indicated an increase in female oviposition rate in response to intensive leg rubbing but failed to find any support for an effect on sex allocation. The overall sex ratio of offspring was slightly male biased but females did not appear to regulate the sex ratio of their offspring.

Wang *et al*., (2005) studied the effect of diamond black moth (DBM), *Plutella xylostella* (Lep.,Plutellidae) male and female multiple mating on fecundity, fertility, and longevity and found that there were no significant differences in the fecundity, fertility, and longevity between the single and twice mated females. Results suggested that DBM females might be monandrous. Multiple mating did not increase male or female mating fitness.

DETERRENTS AND ANTIFEEDANTS

In an investigation into the effects of secondary compounds from tropical plants (*Artocarpus heterophyllus*, *Anacardium occidentale* and *Mimosa pudica*) on the diamondback moth (*Plutella xylostella*), Qin-Wei Quan *et al*., (2004) confirmed the oviposition deterrence and antifeedant

effects in all extracts and observed that the deterrent effect was reduced with time. *A. occidentale* extracts showed continuous oviposition deterrent effect.

Raja *et al*., (2003) analyzed the effects of plant extracts on *Spodoptera litura* (Lepidoptera: Noctuidae) and established that the hexane, diethyl-ether, dichlormethane, ethyl acetate, methanol and aqueous extracts collected from leaves and roots of *Artemisia nilagirica*, and from the leaves of *Acorus calamus, Anisomeles malabarica, Cassia auriculata, Holoptelea integrifolia, Lobelia leschenaultiana, Tarrena asiatica, Pergularia daemia* and *Wedelia calendulacea* showed significant ovicidal, insecticidal and ovipositional deterrent activities.

Eupatorium odoratum [*Chromolaena odorata*] and *Eucalyptus robusta* (JiDong *et al*., 2002) were found to have the greatest oviposition deterrent effects against *Conopomorpha sinensis*. Oil, methanolic seed extract, acetone leaf extract, aqueous seed extract, chloroform seed extract and petroleum ether seed extract of karanj were evaluated and found to act as oviposition deterrents, antifeedants and larvicides against a wide range of insect pests (Kumar and Singh, 2002).

Evaluation of the effect of celangulim (from *Celastrus angulatus* extracts) on the population dynamics of the diamondback moth *Plutella xylostella* (Ming *et al*., 2002) confirmed that celangulim strongly deter the adults from laying eggs and significantly inhibit larval feeding.

Studies on extracts of *Rhododendron molle* as oviposition deterrents and ovicides against *Plutella xylostella* (Lepidoptera: Plutellidae) carried out by Hua (2000) proved that it is an effective deterrent and ovicide against *Plutella xylostella*. Evaluation of the comparative efficacy and protectant ability of powdered and ethanolic extracts of *Dennettia tripetala* fruits, root bark, and leaves in suppressing the oviposition and development of

Callosobruchus maculatus on stored cowpea revealed that the powdered fruits and bark of *D. tripetala* had ovicidal, larvicidal, and insecticidal effects on *C. maculatus* (Adedire and Lajide, 2000).

Aziz and Ismail (2000) tested the effectiveness of three plant oils (*Nigella* sp. *Nigella sativa*, *Boswellia sacra* and, *Cucurbita maxima*) on the bean bruchids (*Bruchidius incarnates*) and were found to possess repellent, oviposition deterrent and protectant effects.

Elhag *et al*., (1999) evaluated methanol and diethyl ether extracts of harmal(*Rhazya stricta*), neem seed kernels (*Azadirachta indica*), cloves (*Syzygium aromaticum*), citrus peel and ramram (*Heliotropium bacciferum*) for their deterrence to oviposition by *Callosobruchus maculatus* on chickpeas and found that both extracts of all materials significantly reduced oviposition on treated seeds.

Evaluation of the effect of aqueous extracts of *Trichilia pallida* leaves and twigs on the development and oviposition of *Tuta absoluta* (Thomazini *et al*., 2000) indicated that the leaf and twig extracts affected insect development, mainly at the larval stage. Methanol extracts of leaves of *Ageratum houstonianum*, *Artemisia brevifolia*, and leaves and drupes of *Melia azedarach*, showed varying degrees of oviposition deterrent effect against *Henosepilachna vigintioctopunctata* (Meena *et al*., 1998).

Plant products (neem seed kernel powder, neem leaf powder and *Lantana camara* leaf powder and two aromatic oils (*Citronella and Palmarosa*) were evaluated against the ground nut bruchid, *Caryedon serratus*, a serious pest of ground nut pods and kernels (Kumari *et al*., 1998). Citronella oil and palmarosa oil gave total protection to groundnut pods by inhibiting oviposition by the bruchid and among the plant powders, *L. camara*

had a good oviposition deterrent activity, but lost effectiveness gradually after one month.

Out of twelve plant extracts evaluated for their oviposition deterrent properties against khapra beetle, *Trogoderma granarium* (Dwivedi and Kumar, 1999) extracts of *Cassia occidentalis* and *Withania somnifera* were effective oviposition deterrents. A methanol extract of *Momordica charantia* leaves strongly deterred Cucurbitaceous feeding beetle species viz., *Aulacophora femoralis*, *A. nigripennis*, *Epilachna admirabilis*, and *E. boisduvali* from feeding (Abe and Matsuda, 2000).

The plant extracts viz., neem (*Azadirachta indica*), arandi (*Ricinus communis*), and karanj (*Derris indica*) [*Pongamia pinnata*], pilu (*Salvadora oleoides*), marva (*Ocimum basilicum*), amaltas (*Cassia fistula*), bluegum (*Eucalyptus globulus*), guava (*Psidium pyriferum*), dhatura (*Datura metel*), and bougainvillea (*Bougainvillea* sp.) applied to sorghum were evaluated as oviposition deterrents against *Tribolium castaneum*. In general, all plant extracts significantly reduced the oviposition of *T. castaneum* on jowar seeds (Lohra *et al*., 2001).

Evaluation of the effects of seed treatment and fumigation of artificially infested cowpea with the volatile oil of air-dried leaves of *Ageratum conyzoides* (Asteraceae) (Gbolade *et al*., 1999) resulted in acute toxicity to adults of the cowpea weevil, *Callosobruchus maculatus*.

The oviposition deterrent and antifeedant activity of 2 formulations containing Neemrich I + oil of *Salvadora oleoides* and Neemrich I + neem extract (Plantmix I and Plantmix II, respectively) were examined against *Phthorimaea operculella* and both the formulations showed oviposition deterrent and antifeedent activity, greater activity than their individual constituents (Sharma *et al*., 1998).

Acetone extracts of *Cassia occidentalis* and *Croton bonplandianus* and pet-ether extracts of *Verbesina encelioides* and *Cassia occidentalis* were effective in deterring oviposition in *Callosobruchus chinensis* (Maheshwari and Dwivedi, 1997).

Studies were conducted to determine the ovicidal and oviposition deterrent properties of acetone, alcohol, benzene, petroleum ether and distilled water extracts of 10 plant species against *Phthorimaea operculella* (Sharma *et al*., 1997) and the results indicated that the efficacy of alcohol extracts was superior to that of other solvents in reducing egg hatch and oviposition.

Evaluation of various materials such as horticultural oils, an insecticidal soap, neem, garlic extract, a sugar ester, and a synthetic insect growth regulator (fenoxycarb) for their ability to inhibit *Cacopsylla pyricola* feeding and oviposition (Weissling *et al*., 1997) indicated that they could be successfully used as oviposition and feeding deterrents.

Spraying the bhendi [okra] crop with various neem products resulted in an oviposition-deterrent effect on females of the pest *Amrasca biguttula biguttula* (Patel and Patel, 1996). Evaluation of some hexane extract of leaf and chloroform extract of seed of *Annona squamosa* as feeding deterrents against adult *Longitarsus nigripennis* (Coleoptera: Chrysomelidae) (Babu *et al* 1996) showed high feeding deterrence.

Applications of 3 concentration of oil-free neem seed extracts (*Azadirachta indica*) to cabbage plants in cages did not deter oviposition by individuals of 3 species of noctuid moths, *Trichoplusia ni*, *Peridroma saucia* and *Spodoptera litura*. 1% crude oil emulsion significantly reduced the proportion of eggs laid by *S. litura* on treated plants. Sprays consisting of highly processed neem seed extracts, used at concentration that provide larval

control, are unlikely to be generally effective as oviposition deterrents to noctuid pests (Naumann and Isman, 1995).

Dilawari *et al*., (1994) indicated that the methanolic extract of *Melia azedarach* effectively checked the fecundity and contributed to the mortality at various stages of the life cycle of diamond-back moth, *Plutella xylostella.* Extracts from calyxes of an alternate host plant, *Hibiscus sabdariffa* exhibited antifeedant as well as oviposition-deterrent activities against *Earias vittella* (Dongre and Rahalkar, 1992).

Various neem, *Azadirachta indica* products were compared with copra oil, palm kernel oil and 0.25% diazinon dust for protection of stored maize against the curculionid *Sitophilus zeamais*. Although copra and palm kernel oil reduced attack by *S. zeamais*, these edible oils were not as effective as neem oil (Cobbinah and Kwarteng, 1989).

Ethanolic extracts of seeds of neem (*Azadirachta indica*), *Caropa procera, Lansium domesticum and Swietenia macrophylla* were highly active feeding deterrents against the southern corn rootworm, while hexane extracts were ineffective as deterrents. (Landis and Gould, 1989).

Vegetable oils, particularly groundnut and palm oils, are known to be effective in controlling some pests of stored pulses (Golob and Webley 1980). Nigerian fish merchants rub groundnut and other vegetable oils on dried fish for protective or cosmetic reasons (Don- Pedro 1989 and 1990). In Senegal, traders in Dakar retail market coat dried fish with vegetable oil to protect it from insect infestation (Wood 1982). However, results from an experiment in the Lake Turkana region of Kenya, in which bottled cod-liver was applied to dried tilapia (*Oreochromis* sp.) at a treatment level of 44 ml/kg, showed that after 45 days storage there was little difference in insect infestation between treated and untreated samples (Walker and Wood, 1986).

Leatemia and Isman (2004) studied the toxicity and antifeedant activity of crude seed extracts of *Annona squamosa* against the lepidopteran pests viz. diamond-back moth, *Plutella xylostella* and cabbage looper, *Trichoplusia ni*. Crude aqueous extracts deterred feeding of 4th instar *P. xylostella* in a leaf disc choice bioassay. Aqueous seed extracts and aqueous emulsion of ethnolic seed extracts were toxic to both species.

Application of oils (ground nut, traditional coconut, industrial coconut, palm, and shark liver oil) against *Dermestes maculatus* on dried trout (*Salmo gairadnerii*) (Don-Pedro, 1989) significantly reduced the development of progeny of *D. maculatus* only at dosages of 56ml/ kg. When *D. maculatus* eggs were assayed against groundnut oil, freshly applied on dried trout surfaces, the LC 50 value was found to be as low as 18.29/ kg. It was observed that absorption of surface oils by fish muscle over time reduced activity against eggs. Generally, the oils were shown to act mainly against eggs and have no direct toxicity against active stages of the insect.

Natural products from parthenium fed (in artificial diet) to *Heliothis zea* were found to be consistently inhibitory. At a dietary concentration of 3.0-mm/kg fr.wt; tetrneurin-A (a parthenolide) reduced larval growth of *H. zea* by 88% relative to controls in a chronic feeding bioassay (Isman and Rodriguez, 1983).

Using the dry fish weight loss, number of live larvae, number of pupae formed and number of live adults as indices of activity, Adedire and Lajide (2000), suggested that *Piper guineense* and *Dennettia tripetala* possess contact toxicity, fumigant, oviposition inhibition, ovicidal and larvicidal activities against *D. maculatus*.

Treatment of dried Tilapia with 0.25, 0.50, 1, and 2 g of neemseed powder per 25 gm of fish (Okorie *et al.*, 1990) affected the oviposition and

the hatchability of different age group of eggs of *D. maculatus* and also killed the adults. Incubation period was prolonged. Larvae did not develop beyond the 2nd instar and 93% of the larvae died by day 30. However, some adverse features of using neem have been observed. Neem powder produces bitterness in taste, which was removed by boiling, and neem oil was observed by Mathan *et al* (1992) to have nauseating, objectionable odour that was picked up by both the packaging and the fish.

The antifeedant and growth inhibitory effects of toosendanin,a limnoid allelochemical from the bark of the trees *Melia toosendan* and *M. azedarach* on variegated cutworm, *Peridroma saucia* were studied using different bioassays by Chen *et al.,* (1995). It was demonstrated that toosendanin significantly deterred feeding of 2nd and 4th instar larvae in diet choice and leaf disc bioassays, respectively. They were also able to prove that toosendanin was a reasonably effective antifeedant against *P. saucia* with a DC50 of 8.μg /cm^2 in the leaf disc choice test.

Evaluation of the efficacy of refined soyabean and crude castor oils for the control of infestations of *Callosobruchus maculatus* and *C. phascoli* in stored chick-pea, *Cicer arietinum* (Pacheco *et al*., 1995) proved castor oil as an effective protectant than soybean oil. No harmful effect was observed on the germination of oil treated seeds.

The potential of four vegetable oils and ten botanical powders in managing the bruchid beetles of legumes, *Callosobruchus chinensis, C.maculatus, C. rhodesianus* were looked in to by Rajapakse and Van Emden (1997) and all four oils tested (corn, ground nut, sunflower and sesame) significantly reduced the oviposition of all three bruchid species at 10ml/kg and also significantly reduced the longevity of adults of *C. maculatus* and *C. chinensis* at this dose. Only corn and sunflower oil caused a significant reduction of longevity of *C. rhodensianus* at 10ml /kg.

Laboratory investigations on the activity of neem leaf and seed extract in water or methylated spirit on *C. maculatus*, *Sitophilus oryzae* and Cylas *puncticollis* (Makanjuola, 1989) showed that the effectiveness of neem is affected by differences in insect behaviour. The extracts were more active as suppressants *of C. maculatus* than *Sitophilus* spp. there was no effect on *C. puncticollis*. All of the extracts tested resulted in a significant reduction in oviposition, percentage egg hatch and percentage adult emergence in *C. maculatus* and in adult emergence of *Sitophilu*s spp.

Two commercially available repellents (oil of clove and citronellol) were found to be effective (Plarre *et al*., 1997) against the webbing clothes moth, *Tineola bisselliella* (Lepidoptera: Tineidae).

Inhibition of larval growth was directly related to concentration of the respective extracts (Villani and Gould, 1985). Out of 78 plant species (24 families) screened for antifeedant activity against the corn wireworm, *Melanotus communis,* five extracts from four families significantly reduced wireworm-feeding damage in a series of choice feeding tests. Two extracts, *Asclepias tuberosa* and *Hedera helix,* exhibited exceptional levels of feeding deterrency. Inspection on the effects of salt treatment of fish on the developmental biology of *D. maculatus* and *Necrobia rufipes* indicated prolonged larval development in both beetles in salted fish and larval mortality was total in *D. maculatus* at salt concentrations of 9.20% and 10.20%. (Osuji, 1975b).

Topical application of different doses of acetone extracts of *Anthocephalus cadamba, Lantana camara, Tectona grandis, Calophyllum* sp. and *Phyllanthus emblica* to the newly moulted last nymphal instar of *Dysdecus cingulatus* resulted in the 6^{th} instars retaining varying degrees of nymphal characters. (Prabhu and John, 1975).

All the four plants viz. *Piper gunieense*, *Cyperus rotundus*, *Dennettia tripetala* and *Capsicum frutescens* were found to be effective in controlling *Dermestes maculatus* on stored, smoked catfish (*Clarias gariepinus* (Adedire et al., 1999).

Piper fruit oil at dosages of 0.125ml/25g fish and 0.150ml/25g, fish were found to be efficient in the control of the development of *D. maculatus* adults and larval stages on tested dried fish (*Clarias* spp.) and was therefore recommended as appropriate dosages for prevention of insect infestation on dried fish (Amusan and Okorie, 2002).

Studies on the mode of action of citrus peel oils (Don-Pedro, 1999) revealed that they are fast-acting fumigant insecticides with possible neurotoxic or anti-respiratory properties. The rapid action of citrus oil fumes was demonstrated by LT50 values of 40.7, 106 h for lime peel oil and 4.6, 14.8 h for d-limonene against *C. maculatus* and *D. maculatus*, respectively.

D. tripetala seed powder showed higher repellency than pyrethrins. Acetone and ethanol extracts were good repellents to *D. maculatus*. Water extracts did not meet the minimum requirement for good repellents (Egwunyenga *et al*., 1998).

The biological action of citrus peel oils was shown to depend on a strong fumigant action (Don-Pedro, 1996 a). Bioassays conducted showed that all the 6 citrus oils tested had vapour toxicity to adults of *C. maculatus*, *Sitophilus zeamais*, and *D. maculatus*. The 24-h LC50 value of lime peel oil (a typical citrus oil) vapour against *C. maculatus* was 7.99 µl/litre which made it 1.5 and 1.6 times less toxic to the smaller *S. zeamais* and the larger *D. maculatus* adults, respectively. When immature stages were fumigated, lime peel oil vapour had 24-h LC50s of 7.8 and 21.5 µ l/litre against eggs of *C. maculatus* and *D. maculatus*, respectively and 9.1, 17.8, 23.1, 23.9 µ litre/litre

against early larvae and pupae of *C. maculatus* and late larvae and pupae of *D. maculatus*, respectively.

Treatment (> 10 ml/mg) against *C. maculatus* or *Sitophilus zeamais*;(>20 ml/kg against *D. maculatus*) with citrus peel oils (lime, tangerine [mandarin] and grapefruit) reduced oviposition or larval emergence through parental adult mortality, but had no residual activity on the eggs or larvae produced by survivors (Don-Pedro, 1996 b). Oil-treated cowpeas (7 ml/kg against *C. maculatus*) or dried fish (28 ml/kg against *D. maculatus*), which caused 100% mortality 1 h after application lost all activity within 24 h, thus confirming the non-residual nature of the effects.

Don Pedro (1985) investigated the effectiveness of powders of the dried peel of orange (*Citrus sinensis*) and grape fruit (*C. paradisi*) using chips of dried cat fish (*Clarias* sp.) and found that orange peel had greater insecticidal and repellency effects than grapefruit peel. Treatment of fish with 14.1% by weight of orange peel poder killed 50% of adult *D. maculatus* after 7 days: a 21.3% treatment killed 99% in the same period. At applications of 15.0 and 18.0% by weight orange peel powder reduced progeny development and slowed larval development. At 18% the number of emerging larvae was reduced by 60% compared with the untreated control. Of the larvae that did emerge only 32.7% and 37.1% of the 18.0% and 15.0% treatments, respectively, developed into F1 adults compared with 87.8% of the larvae from the untreated controls. Subsequent work by Don Pedro (1996a; 1996b) demonstrated that topical toxicity of citrus peel oils was relatively ineffective when compared with activity in the vapour phase. The volatile components possessed activity against *D.maculatus* life stages, eggs being most susceptible and last instar larvae and pupae being least susceptible, though the differences were slight. In the presence of dried fish pieces the activity was greatly reduced as a result of sorption of the volatile components.

Population suppression and toxicity tests were carried out on dried fish pests using the African locust bean plant, *Parkia clappertoniana* (Odeyemi *et al*., 2000). The pod and pulp of *P. clappertoniana* at the rate of 1g, 1.5 g, and 2.0 g for dry powder treatment per 100 g dried fish samples, were toxic to adults and larvae of *D. maculatus* and *N. rufipes*. The population of adults decreased significantly ($P<0.05$) on treated samples. The powder-in-oil treatment had more insecticidal effect on the beetles than the pulp.

Saha and Shajahan (1998) tested the effect of alcoholic extracts of neem and gamma radiation on the 6^{th} instar larvae of *D. maculatus* to investigate their effect on larval mortality, pupation, adult emergence and longevity. 80% larval mortality was obtained at a dose of 19700 ppm of the crude neem extract. Though this dose could not prevent adult emergence, it resulted in deformed adults with reduced life span.

Gakuru and Faua-Bi (1996) compared the effects of essential oils of four plants against *C. maculatus* and rice weevil, *S. oryzae*. Results proved that essential oils had no effect on *S. oryzae*, however, the essential oils of *Eucalyptus citriodora* and *O. basilicum* were more potent against *C. maculatus*.

The study of Lale and Ajay (2000) revealed that clove oil was significantly more toxic to adults and larvae of *Tribolium castaneum* than other oils tested. The period of exposure appeared to be the most important factor to determine the efficiency of extracts rather than dosage (El- Nahal *et al*., 1989).

Ali *et al*., (1983) reported that seeds treated with neem, coconut, mahua, sesame, and palm oil did not permit adult beetles (*C. maculatus*) to lay eggs and thus inhibited the development of subsequent population. Seed oils of *Cassia occidentalis* induced high mortality of bruchid eggs and first

instar larvae than the fresh and dry leaves or ground seeds (Lienard *et al.*, 1993). Several fatty acids (linoleic, oleic, and stearic) present in the oil were responsible for this toxicity. Gupta *et al*., (2000) evaluated the efficacy of different vegetable oils such as castor, mustard, linseed, soybean, coconut, groundnut, and sesame against *S. oryzae*. It was observed that all the oils afforded protection over a period of around 120 days. Among these, mustard and linseed oils were significantly superior in comparison to other oils. According to Shaaya *et al*., (1997), edible oils are potential control agents against stored grain pests like *C. maculatus*, *S. zeamais*, *S. oryzae* and *S. cerealella* on the farm level itself.

Richa *et al*., (1995) have evaluated the effectiveness of essential oils of some plants (basil, geranium, rue, lemon grass, citronella, eucalyptus, and lemon) in protecting faba beans from *C. chinensis*. According to their findings, essential oils of basil and geranium had the greatest insecticidal effect, while oils of lemon grass and eucalyptus were not toxic to adults but showed some effect on oviposition. Insecticidal effect of volatile oils of *Lippia adoensis*, *Cymbopogon citrates*, *Lantana camara* and *Chromolaena odorata* against *C. maculatus* was studied by Gbolade and Adebaye (1995).

Plant oils obtained from cottonseeds, soybean, maize and peanuts act as very good repellents against *S. granaries* in stored wheat (Yun and Burkholder, 1981). Jilani and Su (1983) have demonstrated the repellent effects of turmeric (*Curcuma longa*), neem (*A. indica*) and fenugreek (*Trigonella foenumgracum*) against three species of stored product insects viz. *T. castaneum*, *R. dominica* and *S. granaries*. Results showed that the turmeric powder was the most effective against *S. granaries* and *R. dominica* while only solvent extract was effective against *T.castaneum*. Repellent and growth-inhibitory effect of turmeric oil, sweetflag oil, neem oil, and Margosan-O on red flour beetle, *T. castaneum* were reported by Jilani *et al*., (1988). Their

report indicated that repellency increased with increasing concentration of the oils and Margosan.

Singh and Singh (1991) screened 31 essential oils of plant origin for repellent and insecticidal properties against house fly, *Musca domestica*. The essential oils obtained from *Ocimum gratissimum*, *Thymus serpyllum*, *Illicium verum*, *Myristica fragrans*, and *Curcuma amada* showed 100% repellent activity, and *A.calamus* showed 40% activity.

Malik and Naqvi (1984) have screened seven plant species for their repellent activity against *T. castaneum* and antifeedant activity against *R. dominica*. The best repellent activity was for the rhizomes of *Saussurea lappa* and antifeedant activity for the leaves of *Chenopodium ambrosioides* and for azadirachtin isolated from neem kernal.

Behal (1998) has screened 12 plant oils for their repellent effect against the rice moth, *C. cephalonica*. Study showed that there was a complete repellency for larvae with sweetflag, (*A. calamus*) oil irrespective of its concentration, while with other oils, a concentration dependent repellency was noticed. Petroleum ether extracts of *Cassia tora*, *C. fistula*, and *C. articulata* seeds exhibited more than 80% repellency against *T. castaneum* (Pradeep and Radhakrishnan, 1999).

Sahayraj and Paulraj (2000) observed that *Spodoptera litura* larva was repelled by groundnut leaves treated with *Tridax procumbens* leaf extract and the repellency increased as the concentration of leaf extract increased.

The percent repellency was found to decrease over a long interval of time (Urs and Srilatha, 1990). When essential oil of eucalyptus was used against the rice weevil, *S. oryzae*, 80% repellency was shown after 10 min, 50% after 30 min and after 60 min it decreased to 20% (Ahmed and Eapen,

1986). Malik and Naqvi (1984) also reported a similar time-dependent decrease of repellent property in the case of *T. castaneum*.

Dormusoglu *et al*., (2003) studied the effects of Neem Azal T/S and neem oil on different stages of *Nezara viridula* (L.) (Heteroptera: Pentatomidae) and observed that both products had no significant effect on adults and newly laid eggs. However neem oil was found to be more effective than Neem Azal T/S on nymphs and old laid eggs after 7 and 14 days respectively.

Zabel *et al*., (2002) investigated the effect of neem extract on *Lymantria dispar* and *Leptinotarsa decemlineata* and proved high antifeedency and low toxicity of the plant preparation on *L. dispar* and on 3rd instar larvae of *L. decemlineata*.

Ahmad *et al.,* (2003) conducted experiments on the effects of neem-treated aphids as food/host on their predators and of the three neem preparations sprayed upon eggs. Only neem oil was found to exert a negative impact on the hatching rate of *Cocinella septempunctata* and *Chrysoperla carnea* and the 1st instar larvae *Episyrphus balteatus* proved to be highly susceptible, when feeding 24h. on aphid sprayed with neem kernal water extract.

Ma *et al*., (2000b) studied the biological effects of azadirachtin on *Helicoverpa armigera* fed on cotton and artificial diet and observed high mortality of larvae fed on potted cotton plants (*Gossypium hirtum* L) sprayed with formulated neem extract (3% azadirachtin emulsifiable concentrate). He also found physiological effects such as difficulty in moulting between various instars and abnormal pupae in larvae fed on the artificial diet.

Bruce *et al*., (2004) studied effects of neem oil on oviposition, development, and reproductive potentials of *Sesamia calamistis* and *Eldana*

saccharina Walker and observed low oviposition rates, immature survival, fecundity, and egg viability in the neem treatments and a relatively high persistence of neem oil.

Mathen *et al*. (1992) investigated the insecticidal properties of cashew nut shell liquid and oils of coconut, neem, palm, gingelly, mustard,sunflower, safflower, castor seed, rice bran, ground nut, and hydnocarpus, using dried silver belly (*Leiognathus* sp.). Oils were sprayed on to the packing material, the gunny bags, and on the fish themselves. The authors stated that mustard oil was observed to be the best insect repellent, the treated sample remaining insect free for 40 days, followed by hydnocarpus, sunflower and cashew. Unfortunately, little data was given, particularly regarding application rates and the species of insect used in the experiment.

Peppers (*Capsicum* sp.) are used traditionally in Africa as a means of repelling blowflies but there is no evidence to suggest a use on dried fish. Pepper is used domestically as a preservative for prawns in Kerala, India. When cured prawns were sprinkled with 1.5% by weight of pepper powder and stored in a mite-infested godown (store) they remained insect free for as long as the pepper smell persisted, about 7 weeks. (Pillai, 1957).

Wood *et al*., (1987) studied the effect of salt on the susceptibility of dried whiting to attack by dermestid beetles. The maximum weight losses in unsalted fish varied between trials and ranged from 15 to 41%. Increases in salt content above the natural 2% level to 5% did not give any marked protection against insect infestation. A salt content of greater than 9% largely protected the fish from damage, reduced weight losses to less than 10% and greatly inhibited insect development.

Experiments with salted dried fish (the freshwater fish, *Roccus chrysops* being used) showed that a salt content of 13% or more prevented the

development of infestation by *D. maculatus* from eggs. The adverse effects were reduction of larval survival and retardation of larval development rather than reduction of egg viability. The results suggested that eggs laid in the crevices of salted fish will hatch but that the larvae will mostly perish (Mushi and Chiang, 1974).

CHAPTER II

MATERIALS AND TECHNIQUES OF STUDY

MATERIALS AND METHODS

Maintenance of stock culture

A stock culture of *N. rufipes* was maintained in glass troughs measuring 25cm x 25cm x 11.5cm. in the laboratory on a diet of dried sardine. The original sample was taken from the fish drying centers in Puthiyappa, Calicut. The duration of the present study was from 2004-2006

Biology

Eggs laid by freshly mated females were enclosed in separate specimen tubes (60 in number) measuring 10.5cm X 2.5cm.The tubes were closed with cotton plugs which were soaked with water every 24 hours to provide moisture for the developing larvae. Dried sardine was provided as food and was changed every second day. Observations were made at 24-hour intervals and larval moults were recorded by noting the presence of exuviae.

Fecundity

Freshly emerged males and females were paired in specimen tubes (measuring 10.5cm X 2.5cm). The mated females (20 numbers) were kept individually in the specimen tubes which were closed with cotton plugs. The cotton plugs were soaked with water every day to provide free water for the beetles. Water is provided, as according to Dick (1937) and Taylor (1964) females with access to free water lay more eggs than with those without and oviposition period is also restricted. Dried sardine (approximately 20 g) was provided as food and oviposition medium. It was removed at 24 hr. intervals and the eggs were counted and fresh pieces of dried fish were provided. To study multiple mating, male and female were kept together till death and for double mating the male was removed after a single mating. When the females

stopped laying eggs, they were mated once again after a gap of 15 days with a freshly emerged male.

Preliminary studies indicated that the females that have stopped laying eggs sometimes resumed egg laying after a maximum gap of 12 days after the first mating. Hence, the gap of 15 days between the two mating.

Longevity

Freshly emerged male and female *N.rufipes* (20 numbers each) were kept in individually in specimen tubes (measuring 10.5cm X 2.5cm). Dried sardine provided as food was changed every 2nd day. The cotton plugs were soaked with water after every 24 hours to provide moisture for the insects. Observations were made every 24 hours and mortality was recorded.

Courtship and Mating behaviour

The working area was lined with a clean piece of filter paper. A petri dish (10cm x 2cm) was kept upside down on the filter paper. This was used as the observation arena. A virgin male was introduced under the inverted petridish and was allowed to acclimatize to the surroundings for at least 5 minutes. A virgin female was then introduced. The ensuing sequences of events were carefully observed. If the male and the female did not interact after 2-3 minutes, a different pair was tried. Five such trials were made.

Preparation of plant extracts

Water extracts

Leaves were dried under the shade for 1 week and then powdered in a grinder. 25 gm leaf powder was mixed in 100 ml water. Boiled for 2-3 minutes in a 1000 ml conical flask. Strained it with muslin cloth. Squeezed gently. The residue in the muslin cloth was then mixed in 50ml water and

boiled for 2-3 minutes. It was again strained and made up to 100 ml. Extracted three times with 100, 50, and 50 ml water to make it 150 ml stock solution (Devasahayam and Leela, 1997). Dry fish pieces were immersed in the filtrate of required concentration for 15-30 minutes, dried in the sun and then supplied to various instars and adults of *N. rufipes*. 10 larvae/adults were kept individually in specimen tubes measuring 10.5cm X 2.5cm along with the treated dried fish. Experiments were replicated three times. The control experimental set up contained dried fish soaked in water and dried.

Alcoholic solution

100 ml leaf powder was mixed in 300 ml alcohol, stirred well. If it exists as slurry then added more alcohol. Covered and kept it overnight. Filtered it into a conical flask. Extracted thrice with 150 ml. Combined filtrate was kept in a water bath. Evaporated to dryness. It was taken as 100%. Took 0.5%, 1%, and 2% as per the requirement (Devasahayam and Leela, 1997).

Dry fish pieces were immersed in the filtrate of required concentration for 15-30 minutes. Dried in the sun and then supplied to various instars and adults of *N. rufipes*. 10 larvae/adults were kept individually in specimen tubes measuring 10.5cm X 2.5cm along with the treated dried fish. Experiments were replicated three times. The control experimental set up contained dried fish soaked in absolute alcohol and dried.

Spices oils

Crude Lemon Grass (LG) oil was collected from Waynad .The crude Lemon Grass oil was fractionated from the Dept. Of Chemistry, University of Calicut in to Lemon Grass A (LG A) oil and Lemon Grass B (LG B) oil.

The other oils such as Cinnamon Leaf oil, Clove bud oil, Black Pepper oil and Turmeric oils were supplied by Synthite Cochin, India.

Spices oils were diluted to 10%, 5%. 2%, 1%, 0.5% and 0.25% concentrations in ethanol and cotton balls approximately 0.5 to 0.75 cm in diameter were wetted with 10 drops each of the diluted solution (each drop is approximately 0.05 ml) The cotton balls were than introduced in to the specimen tubes (measuring 10.5cm X 2.5cm) containing egg /larvae or adult and dry fishmeal. 10 larvae/adult were housed individually in the specimen tubes. Experiments were replicated three times. The cotton ball was separated from the larvae and food by 1mm wire gauze. Mortality of the respective stages of *N. rufipes* was recorded after 24, 48 and 72 hours after treatment. In the control set up the cotton balls were wetted with absolute alcohol diluted with water as per the concentration.

Survey

Population study of the various insect pests were not possible because the pests were located in different area (on bamboo poles, under coir mats, on and inside gunny bags, in the sand, in the waste dumped outside the sheds and also inside the fish that are heaped in the sheds), a uniform method of sampling is not possible and as pooling data from different sampling methods may increase sampling errors it was not attempted. The abundance of each pest species was estimated from an approximate visual count. The survey was conducted in almost all the Coastal districts of Kerala viz., Kasaragode, Kannur, Kozhikode, Malappuram, Trichur, Ernakulam, Alleppey, Kollam, and Thiruvanathapuram. A representative center was selected for each district based on the scale of fish drying being carried out. Monthly sampling and collection were carried out in Thiruvanthapuram, Kollam, Alleppey, Ernakulam and Trichur, Kannur and Kasaragode at 6 month intervals, while it was fortnightly in Malappuram and Kozhikode.

Experiments to determine presence of insecticides in sand

To test the presence of insecticide in sand where fish were dried, sand collected from different drying centres was kept in a glass trough measuring 25cm x 25cm x 11.5cm. *N. rufipes* 2nd instar larvae were introduced into the sand. Dried fish in small pieces were supplied as diet. In a separate control experiment clean sand from the beeches (from location on the beeches were fish are not dried) are also used a separate glass trough. Larval death after 24 hours was noted.

Statistical analysis

Pearson's chi-square test (χ^2) was carried out to analyse data on developmental biology. In our case, we need to test whether the environmental factors (temperature & photo period) are influencing on developmental periods of IP and various instars. Temperature, photo period and developmental periods are categorical variables and hence a most appropriate test for independence is χ^2-test of independence. For each category, we formed a two-way table (called contingency table) which gives the observed frequencies of different levels of two factors (eg. For each particular photo period, we formed a two-way table of temperature against developmental periods of incubation period). Using the assumption of independence, the software (Statistica) evaluates the expected frequencies and compute

$$\chi^2 = \sum \left(\frac{(O-E)^2}{E} \right)$$

where O stands for an observed frequency, E stands for the corresponding expected frequency and $\sum$ stands taking the sum total of all computed values in the bracket.

The software also gives p-value of the test and raw percentages. If p-value is less than 0.01, the χ^2-value is highly significant which means that the two factors (raw factor and column) are highly associated. If p-value is greater than 0.01, but less than 0.05, the χ^2-value is significant at 5% level, which means that the two factors (raw factor and column) are associated at 5 % level. If p-value is greater than 0.05, the χ^2-value is not significant which means that the two factors (raw factor and column) are independent (no significant association is observed). In significant cases the raw percentages interpret the way the factors are associated.

Toxicity data were analysed by Probit analysis (Finney, 1971). Statistica '99 version was used to carry out all statistical analysis.

CHAPTER III

DEVELOPMENTAL BIOLOGY

DEVELOPMENTAL BIOLOGY

Results

(Larval development at 32.5^{0}C and photoperiod L:D 12:12)

The Egg

The egg of *N. rufipes* has a mean length of 1.5 mm and 0.30 mm width (Plate I, photograph 1). It is tapered and tips are pointed. It is smooth, shining, translucent, and is glued in place. Four eyespots become visible after 24 hours of incubation. The eggshell is torn open by the wriggling movements of the larvae. The incubation period lasts for a mean of 2,13 days (range 2-3 days) (figure 1)

First instar

The first instar larva is delicate, hairy, and pinkish-white (Plate I, photograph 2). Larvae are negatively phototropic. The 1st instar lasts for a mean of 3.19 days (range 3-4 days) (figure 1)

Second instar

Whitish-pink, more hairy, voracious feeder, move fast and prefer crevices (Plate I, photograph 3). The larva eats through the flesh of dried fish resulting in tunnel formation with the remnants of the food and the faecal matter forming the roof of the tunnel. The 2nd instar lasts for a mean of 6.05 days (range 4-7 days) (figure 1)

Third instar

Pinkish-brown body (Plate I, photograph 4). Dorsal side darkly pigmented than the ventral. Hairs are more prominent, and body is narrow and

elongated. The third instar larva eats voraciously and makes holes and tunnels during the process of eating. The 3rd instar lasts for a mean of 6.05 days (range 5-10 days) (figure 1)

Fourth instar

The larva is brownish and hairy (Plate I, photograph 5**)**. The head capsule (Plate I, photograph 6) and the cerci (Plate I, photograph 7) are prominent and dark brown in colour). Larvae were not observed to bore into the bone or skull of dried fish, although larval and adult individuals were often found in open cavities of the skull during surveys of infestation in the field. Following the completion of feeding, full-grown larvae infesting the dried fish migrate from the oily part where they develop and seek a dark, dry spot in which to build the cocoon. The 4th instar lasted for a mean of 6,03 days (range 5-7 days) (figure 1)

The Pre-pupa and pupa

After the fourth instar, the larva contracts in length, the body consequently becoming more robust (Plate I, photograph 8), spins a cocoon and form a pre-pupa (Plate I, photograph 9). The cocoon was completed within a mean of 2 days, and is formed either by filling in the open boundaries of the crevice chosen for pupation or constructing a cocoon attached to the glass vial around the larva with a wall of white substance which is regurgitated from the mouth of the larva in frothy droplets and mixed with the faecal matter and pieces of the fish meal. If disturbed during cocoon building, the larva remained motionless for several minutes. In case the wall of the cocoon was broken, the larvae in the early pre-pupal stage rebuild another complete pupal chamber. In some cases, the larva does not reconstruct the cocoon, but instead goes through the pre-pupal and pupal stages outside the cocoon in an exposed state. The pre-pupal skin is cast and it is transformed

into the pupa. The pre-pupal stage lasts for a mean of 3.26 days (range 2-5 days) (figure 1)

The pupa (Plate II, photograph 10) is restricted in movement to wriggling of the abdomen. The shriveled cast skin of the larva is usually attached to the tip of the abdomen of the exposed pupae (Plate II, photograph 11, 12). The partial sclerotization of the mandible is seen (Plate II, photograph 13). The pupal stage lasts for a mean of 5.87 days (range 5-6 days) (figure 1)

The total development cycle was completed in a mean of 32.04 days (range 29-36 days). The freshly emerged adults (Plate II, photograph 14) were generally creamy white, the skin darkening later into a brownish black, then to metallic blue within 1-2 days. The male (Plate II, photograph 15) is comparatively smaller in size than the female (Plate II, photograph 16). Eclosion is by breaking the wall of the cocoon.

Effect of different temperature and photoperiodicity on biology

25°C, L: D 12:12

10% of the eggs did not hatch and 55% of the eggs hatched on the 2nd day (range-2-5 days) (Table 1). 42.59% larval mortality was recorded and 12.96% each; of the 1st instar larvae moulted into the 2nd instar on the 6th and 7th day (range 3-30 days) (Table 2). 25.81% of the larvae moulted in to 3rd instar on the 11th day and the 2nd instar mortality at this temperature was 35.48% (range 6-14 days) (Table 3). 25% of 3rd instar larvae moulted in to the 4th instar on 11th day where 15% larval mortality was recorded (range 9-14 days) (Table 4). 76.47% of the larvae died on the 1st day and the rest by the 42nd day and no larvae moulted into pre-pupae (Table 5). Temperature influenced the development of all stages ($p=0.00000$).

30°C, L: D 12:12

5% of the eggs did not hatch and 78.33% of the eggs hatched on the second day (2-3 days) (Table 1). 7.02 % 1st instar larvae died and 70.18% of the larvae moulted in to the 2nd instar on the 6th day (range 3-6 days) (Table 2), whereas 7.55% larval mortality was recorded and 35.85% of the 2nd instar larvae moulted into the 3rd instar on the 6th day (range 4-8 days) (Table 3). 36.73% larvae completed the 3rd instar on the 8th day and no mortality was observed (range 5-10 days) (Table 4). 53.06% larvae completed 4th instar on the 6th day. No mortality was observed in this instar and all the individuals moulted into pre-pupae (range 5-8 days) (Table 5).

No pre-pupal death was observed and 48.98% of the pre-pupae pupated on the 6th day (range 6-8 days) (Table 6). 48.98% of the adults emerged on the 12th day of pupation (range 8-18 days) (Table 7). Temperature influenced the development of all stages (p=0.00000).

32.5°C, L: D 12:12

A maximum of 86.67% eggs hatched on the 2nd day and the rest on the 3rd day (range 2-3 days) (Table 1). 1.67% of 1st instar larvae died and 86% of the surviving larvae moulted in to the 2nd instar on the 3rd day (range 3-4 days) (Table 2). 5.08% of the 2nd instar larvae died and 37.29% of the larvae moulted in to the 3rd instar on the 6th day (range 4-8 days) (Table 3). 67.86% of 3rd instar larvae moulted into 4th instar on the 5th day (range 5-10 days) (Table 4). 59.26% of the 4th instar larvae moulted in to the pre-pupae on the 6th day and there was no mortality (range 5-7 days) (Table 5). No pre-pupal death was observed and 68.52% of the pre-pupae moulted in to pupae on 3rd day (range 2-5days) (Table 6). 87.04% of adult emerged from the pupae on 6th day (Table 7). Temperature influenced the development of all stages (p=0.00000). (Fig. 1)

35°C, L: D 12:12

While 90% of the eggs hatched on the 2nd day (Table 1), 10% of the eggs remained unhatched at 35°C. Percentage hatch was influenced by temperature (p=0.000147). A maximum of 81.48% larvae moulted into the 2nd instar on 3rd day (range 2-3 days) (Table 2). No larval mortality was observed in the 2nd instar and 36.73% of the larvae completed the 2nd instar on the 8th day (range 4-8 days) (Table 3). No mortality was observed and 83.67% of the 3rd instar larvae moulted into the next instar on the 5th day (range 4-7 days) (Table 4). At this temperature all the 4th instar larvae died (Table 5). Temperature was found to influence the development of all stages (p=0.00000).

25°C, L: D 00:24

6.67% of the eggs did not hatch and 66.67% of the eggs hatched on the 2nd day (range 2-4 days) (Table 1). 25% of the 1st instar larvae died and 23.21% moulted in to the 2nd instar on the 6th day (range 3-21 days) (Table 2). 11.90% of the 2nd instar larvae died and 30.95% of the larvae moulted into the 3rd instar on 11th day (range 6-13 days) (Table 3). 38.46% of the larvae completed the 3rd instar on 8th day (range 8-12 days) (Table 4). No 4th instar larvae moulted into pre-pupae and all of them died within 40 days (Table 5).

25^0C, L: D 24:0

13.33% of the eggs did not hatch and 35% of the eggs hatched in to the 1st instar on the 2nd day (range 2-5 days) (Table 1). While 53.85% of the 1st instar larvae died, 9.62% of the larvae moulted in to the 2nd instar on the 8th day (range 4-29 days) (Table 2). 25% of 2nd instar larvae moulted into the next instar on 15th day (range 7-17 days) (Table 3). 3rd instar larval mortality was 45% and a maximum of 20% larvae moulted in to the 4th instar on the

12th day (range 9-16 days) (Table 4). No 4th instar larvae moulted into pre-pupae and all of them died within 51 days (Table 5).

30°C, L: D 00:24

3.33% eggs were unhatched and 73.33% of the eggs hatched on the 2nd day (range 2-3 days) (Table 1). 8.62% of the 1st instar larvae died and 75.86% of the larvae completed the 1st instar on 5th day (range 3-5 days) (Table 2). No mortality was observed and 32.69% and 30.77% of the 2nd instar larvae moulted in to the next instar on the 6th and 7th day respectively (range 4-8 days) (Table 3). No mortality of 3rd instar larvae was recorded and 38.46% of the larvae completed the 3rd instar on 8th day (range 5-9 days) (Table 4). 53.06% of the 4th instar larvae moulted into pre-pupae on the 6th day (range 5-8 days) (Table 5). No pre-pupal mortality was observed and 36.54% of the pre-pupae moulted into pupae on the 5th day (range 5-8 days) (Table 6). No pupal death was recorded and 26.92% of the adults emerged on the 9th day (range 7-12 days) (Table 7).

30°C, L: D 24:0

8.33% eggs did not hatch and 63.33% eggs hatched on 2nd day (range 2-3 days) (Table 1). 14.55% 1st instar larvae died and 56.36% of the larvae moulted in to the 2nd instar on the 6th day (range 2-7 days) (Table 2). 2nd instar larval mortality was 10.64% and 34.04% of the larvae moulted in to 3rd instar on the 7th day (range 5-8 days) (Table 3). No larval mortality was recorded and 38.10% of the 3rd instar larvae moulted in to the next instar on the 8th day (range 5-10 days) (Table 4). None of the 4th instar larvae died and 33.33% each of the larvae moulted in to pre-pupae on the 6th and 8th day (range 2-8 days) (Table 5). No pre pupal mortality was observed and 33.33% of the pre-pupae moulted in to pupae on the 5th day (range 3-6 days) (Table 6). Majority of the adults (64.29%) emerged on the 12th day and no

pupal death was recorded (range 8-13 days) (Table 7). The incubation period and duration of all the larval stages were significantly influenced by all temperature and photoperiod combinations (p= 0.00000).

Discussion

This is the first report on the biology of *N. rufipes* - a major pest of dried fish - from India. The only earlier study on the biology of this pest was carried out in the early 20th century by Simmons and Ellington (1925) with smoked pork as diet, which includes only the description of the morphology of *N. rufipes* larvae and does not mention the details of the larval development.

The only pest of dried fish whose biology has been studied in detail is of *Dermestes maculatus* (Osuji, 1975d), in which case copulation usually took place immediately after the adults were paired and eggs were usually laid within 12-40 hrs. In the case of *N. rufipes* though the copulation occurred immediately after the pairing of adults, there was a pre-oviposition period of 2 days.

Eggs were occasionally laid singly, usually in batches of 4,6, and 8 (Osuji, 1975d) by *D. maculatus*. *N. rufipes* laid eggs singly or in batches of varying numbers with a maximum of 31 eggs in a batch. The incubation period of *N. rufipes* in the present study was found to be 2-3 days at 32.5^{0}C L:D 12:12 (Table –1), which is similar to the result obtained by Simmons and Ellington (1925) in the same species, while in *D. maculatus* it was a mean of 2 days (Osuji, 1975d).

The 1st, 2nd and 3rd instars of *N. rufipes* were completed within a mean of 3.11, 6.05 and 5.5 days respectively in the present study at 32.5^{0}C L: D 12:12, while it was 2, 2 and 7 days for the 1st, 2nd and 3rd instars respectively, in *D. maculatus* (Osuji, 1975d).

The 4th instar larvae of *N. rufipes* contracts in length and become more robust, spins cocoon any where on a dry substratum, including dried fish and form pre-pupa. Contrary to this, the last instar larvae of *D. maculatus* excavate a pre-pupal chamber in the hard, intact dried fish to get encased in the fish and the larval skin was subsequently hardened in to a dark brown pupal case (Osuji, 1975d). This difference in pupation sites may be an adaptation to reduce competition, as both *N. rufipes* and *D. maculatus* co-exist in the field. In *N. rufipes*, pupation occasionally takes place without the protection of the cell as was observed in *D. maculatus* also (Osuji, 1975d).

Osuji (1975d) observed that in *D. maculatus*, pre-pupal period was a mean of 4.9 days, which was found to be comparatively higher than what was observed in *N. rufipes* (3.26 days), in the present study. Whereas, the duration of pupal stage in *D. maculatus* (5.5 days) was found to be almost similar to that observed in *N. rufipes* (5.87 days).

Total larval developmental period in *N. rufipes* at 32.5^0C L: D 12:12 (32.04 days) was comparatively lower than what was reported in *D. maculatus* (33.5 days) by Osuji (1975d). Ashman (1967) had observed that development of *N. rufipes* on copra alone was relatively slow, where the larval and pupal periods were 67 days at an optimum 30^0C and 80% RH and that the addition of protein in the form of dried fish meal to copra reduced this development time to 43 days. But in this study when *N. rufipes* was reared on a diet of dried fish alone at 30^0C and 60% RH, the duration of larval development was still lower.

A sex ratio with a predominance of females was obtained in this study, which would favour the multiplication of the species, by increasing the number of ovipositing individuals. Coombs (1979) reported wide spread cannibalism by larvae and adults of *D. haemorroidalis* and *D. peruvianus*, a

phenomenon, noted only in the absence of food in *N. rufipes*, in the present study.

Although, *N. rufipes* could complete their development cycle in 6 instars compared to 7 in *D. maculatus* (Osuji, 1975d*)*, the almost similar time taken by the larvae of both species to complete the larval development may be the reason for their intense competition in the field.

Comparison of effect of different temperatures (25, 30, 32.5 & 35^0C) at L:D 12:12 on developmental biology

The use of lethal temperatures, both high and low, for insect pest destruction is of importance in many countries. The purpose of this method is not the actual destruction of the pests but the drastic retardation of development following the reduction of the metabolic rate.

It was found that, out of the four different temperatures tested (25, 30, 32.5 and 35°C) at L: D 12:12 photoperiod, 32.5°C was found to be the most suitable temperature. At this temperature all the eggs hatched within 2-3 days and the mortality of the different instars was comparatively low than at all the other three temperatures (25, 30 and 32.5^0C). The influence of temperature and photoperiod on the larval development was found to be significant.

Though, majority of the eggs hatched within 2 days at 35°C, it took longer for all the eggs to hatch at 25°C (2-5 days). But the percentage of egg mortality was same (10%) at both the temperatures, while at 30 and 32.5°C all eggs hatched within 2-3 days and the egg mortality was only 5%. The above results emphasize the suitability of 30 and 32.5°C for optimum egg hatch. But in *Dermestes ater* reared on dried fish, incubation period was 4-5 days at 25, 30 and 35^0C (Coombs, 1981), which is longer than that observed in the present study.

Only 1.67% of the 1st instar larvae were found dead at 32.5°C in contrast to 42.59% death registered at 25°C. The surviving 1st instar larvae moulted in to the 2nd instar within 3-4 days at 32.5°C, whereas at 25°C, a prolonged duration of 3- 30 days was required for the surviving 1st instar larvae to moult in to the next instar. Though the 1st instar larvae moulted in to the next stage within 2-3 days at 35°C, the mortality rate was significantly higher (9.26%) than that at 32.5°C (1.67%).

The very high mortality (42.59%) of 1st instar and the longer period (3-30 days) needed for all the individuals to complete the 1st instar at 25°C showed that, *N. rufipes* will find it difficult to maintain its population when this temperature prevails in the field during the rainy months.

Although no 2nd instar larvae died at 35^0C when compared to 3.57% mortality at 32.5^0C, in the later instars mortality was much higher at 35^0C and all the 4th instar larvae died without moulting into pre-pupa.

All the 3rd instar larvae entered the 4th instar within 4-7 days at 35°C, whereas it took 4-10 days at 32.5°C. Except for the relatively higher mortality, a tremendous increase in the growth rate of the surviving larvae was observed at 35°C. Larval mortality of 3rd instar larvae was relatively higher at 25°C (15%) than at 32.5°C and the larval duration was significantly higher at 25°C (9-14 days) than at any other temperatures tested. This prolonged duration and high mortality shows that this temperature (25°C) does negatively influence growth, which is an indicator that larval development at this temperature is not the normal one, which is also reflected in the high mortality at this temperature.

All the surviving 4th instar larvae died and no pre-pupae were formed at 35°C and 25°C, whereas no 4th instar larval mortality was recorded at 32.5°C and 30°C.

Pupation occurred only at 32.5°C and 30°C. Maximum adult emergence (87.04%) occurred on the 6th day at 32.5°C, where the pupal duration was recorded as 5-8 days, whereas at 30°C, the maximum adult emergence (48.98 %) was recorded on the 12th day and the total pupal duration was 8-18 days. The results indicate that higher temperatures does bring about faster development of the larvae.

Comparison of effect of different photoperiods (L:D 24:00, 00:24 and 12:12) at 25 and 30^{0}C on developmental biology

When the effect of photoperiods at the two temperatures 25 and 30^{0}C were compared, L: D 00:24 was observed to be the best photoperiod for completion of larval development at both temperatures. Significantly high percentage of death (25%) and prolonged 1st instar (3-21 days) was recorded at 25°C, L: D 00:24 photoperiod combination while a considerably low percentage of 1st instar larval death (8.62%) and a very short duration of 4-5 days was recorded at 30°C, L: D 00:24 photoperiod combination.

2nd instar larval mortality was low (11.90%), and the larval duration continued to be prolonged (6-13 days) at 25°C, L: D 00:24 photoperiod regime, as in the case of the 1st instar, whereas no 2nd instar larval death was observed and all the larvae moulted in to the 3rd instar in a comparatively shorter period of 4-8 days at 30°C; L: D 00:24. A significant increase in the larval mortality (21.62%) and increased larval duration (8-12 days) were recorded in the 3rd instar larvae of the beetle pest at 25°C, L: D 00:24, while there was no 3rd instar larval death and all the individuals completed the instar within a comparatively shorter duration (5-9 days) at 30°C; L: D 00:24 photoperiod. All the 4th instar larvae died at 25°C, L: D 00:24, in contrast to the results obtained at 30°C; L: D 00:24 photoperiod combination, where all

the 4th instar larvae moulted in to pre-pupae within a comparatively short period of 5-8 days.

No pupal death was recorded and the adults emerged within 7-12 days after pupation at 30^0C, 00:24. These results confirm the adaptation of the insect to develop in a photonegative environment and that warmer temperature is ideally suited for its normal development.

The failure to pupate at 25°C and 35°C in the laboratory explains the negligible presence of *N. rufipes* at these temperatures in the field. At Ibadan, Nigeria, Osuji (1974b) also observed that although *D. maculatus* was the predominant pest on a range of species of dried fish, *N. rufipes* was also rather abundant at particular times of the year. Both species were more prevalent during the warm dry seasons between October and March, when temperatures rise to above 30^0C, than in the cool wet period between May and July when the temperatures reach 26^0C. But Odeyemi (1997) had observed that at high adult population and moisture content *N. rufipes* become extinct and *D. maculatus* dominates a mixed culture and that at a temperature of 20^0C, *D. maculatus* outcompetes *N. rufipes*, while at 32.5^0C both species co-exist. The author has also observed low occurrence or absence of *N.rufipes* relative to *D. maculatus* in commercial dried fish areas with temperature of 20 to 30^0C. But during the course of the present study *N. rufipes* was observed to dominate in the field throughout the year with high population during the summer months when the temperature was around 32.5^0C, and *D. maculatus* population was very low at this temperature.

In conclusion, it was observed that development was fast and mortality was lower at 32.5^0C, L: D 12:12, than at any other temperature-photoperiod combination. Development at all photoperiods at 25^0C was prolonged and mortality high, which indicates the unsuitability of this temperature for normal development of *N. rufipes*.

Influence of Incubation period on the developmental stages (at 25 and 30°C; L: D 00:24, L: D 24:00 and L: D 12:12)

Result

25^0C, L:D 0:24

50% of the 1st instar larvae, which had an incubation period of 3 or 4 days died on the day of emergence, while 50% completed the 1st instar on the 14th day if they had an incubation period of 4 days (Table 8). 42.86% of 2nd instar larvae moulted into the next instar on the 11th day and no larvae died if they had an incubation period of 2 days (Table 9). Majority (42.86 %) of those 3rd instar larvae that had an incubation period of 3 days completed the instar on the 8th day (Table 10). 17.39 % of those 4th instar larvae died on 36th day, if they had an incubation period of 2 days and no pre-pupae were formed (Table 11). Significant influence of incubation period on all instars was observed (p=0.0000).

25^0C, L:D 24:0

75% of the 1st instar larvae died and the remaining 25% completed the 1st instar on 12th day if they had spent 4 days in incubation (Table 8). 1st instar mortality was 45.45% if those larvae had consumed 2 days in incubation period and 18.18% of the surviving larvae moulted into the next instar on 6th day. No 2nd instar larval mortality was observed if they had expended 2 days in incubation and 27.27% of the surviving larvae completed the 2nd instar on the 15th day (Table 9). 3rd instar larval mortality was 57.14% and 28.57% of the larvae moulted in to the 4th instar on the 12th day if they had spent 3 days in incubation (Table 10). All the 4th instar larvae that had an incubation period of 2 days died on the 40th and 43rd day and none of them moulted into pre-pupae (Table 11). Significant influence of incubation period on all instars was observed (p=0.0000).

25°C, L: D 12:12

If the 1st instar larvae had spent 2 and 3 days in incubation, 45.4% and 36.8% mortality respectively were observed on the first day itself (Table 8). 50% of the larvae completed the 2nd instar on the 11th day if they had an incubation period of 3 days (Table 9). 44.44% of those larvae which had an incubation period of 3 days completed the 3rd instar on the 11th day itself (Table 10). 75% of the 4th instar larvae died if they had an incubation period of 2 days and 25% died on the 38th day and none moulted into pre-pupae (Table 11). Significant influence of incubation period on all instars was observed (p=0.0000).

30^0C,L:D 0:24

Majority of those larvae (79.55%) which had an incubation period of 2 days completed the 1st instar on the 5th day (Table 8). 2.38% of the 2nd instar larvae died and 36.36% of the larvae completed the 2nd instar on 6th day if they had an incubation period of 3 days (Table 9). 39.02% of the 3rd instar larvae moulted into the next instar on the 8th day when they had an incubation period of 2 days (Table 10). 56.1% of 4th instar larvae that had spent 2 days in incubation moulted in to pre-pupa on the 6th day (Table 11). 66.67% of the pre-pupa moulted into pupa on 6th day if they had spent 3 days in incubation (Table 12).

30°C, L: D 24:00

65.79% of the larvae completed the 1st instar on the 6th day, if they had an incubation period of 2 days (Table 8). Those 2nd instar (39.39%) larvae that had an incubation period of 2 days completed the second instar on the 7th day (Table 9). 38.71% of the 3rd instar larvae that had an incubation period of 2 days moulted into 4th instar on the 8th day (Table 10). If the incubation period was 3 days, 45.45% of those 4th instar larvae moulted in to pre-pupa on the 7th

day (Table 11). 54.55% of pre-pupa that had an incubation period of 3 days moulted into pupae on the 6th day. 38.71% of those pre-pupae, which had expended 2 days in incubation, moulted into next instar on the 8th day (Table 12).

30°C, L: D 12:12

If the incubation period was 2 days, 8.51 % of those 1st instar larvae died and 70.21% of the surviving larvae completed the 1st instar on 6th day (Table 8). 50% of the 2nd instar larvae which had an incubation period of 3 days moulted into 3rd instar on the 6th day (Table 9). 44.44% of 3rd instar larvae moulted into the 4th instar on the 6th day when they had an incubation period of 3 days (Table 10). No 3rd instar died at this temperature and photoperiod combination. Irrespective of the incubation period, no 4th instar larval mortality was observed, and 55% of the larvae completed the 4th instar on the 6th day if they had spent 2 days in incubation (Table 11). 66.67% of pre-pupae moulted into pupae on 6th day if they had an incubation period of 3 days (Table 12). Significant influence of the incubation period on the moulting of the pre-pupal instar was observed.

Influence of duration of development stages on subsequent instars at 30°C; L: D 00:24

Results

Influence of incubation period on the subsequent instars

79.55% of the 1st instar larvae that had an incubation period of 2 days moulted into the 2nd instar on the 5th day. No significant influence of the incubation period on the 1st instar of *N.rufipes* was observed (p=0.145475) (Table 13). No mortality was observed and 36.36% of the larvae completed the 2nd instar on the 6th day if they had spent 3 days in the incubation period.

Incubation period did not influence the mortality or moulting of the second instar larvae. (p=0.643403) (Table 14).

39.02% of the 3rd instar larvae, which had spent 3 days in the incubation period moulted into the 4th instar on the 8th day. The influence of incubation period on 3rd instar larvae was insignificant (p=0. 958939) (Table 15).

56.10% of the 4th instar that had expended 2 days in the incubation period moulted into the pre-pupal stage on the 6th day. The time spent in the incubation period did not influence 4th instar duration (p=0. 920977) (Table16).

Those pre-pupae (54.55%) that had spent 3 days in the incubation period moulted into pupae on the 6th day. The effect of incubation period on 4th instar larval period was insignificant (p=0. 0953750) (Table 17).

Majority of the pupae (36.36%) that had spent 3 days in the incubation period moulted into adults on the 9th day. The time spent in incubation period did not influence the emergence of the adults (p=0.664713%) (Table 18).

Influence of duration of 1st instar on the duration of subsequent instars

77.78% of those larvae that had taken 5 days to complete 1st instar completed the 2nd instar on 6th day and no larval death was observed. The time spent in the 1st instar significantly influenced 2nd instar duration (p=0.035233) (Table 19).

If the larvae had spent 4 days in the 1st instar, 55.56% of those 3rd instar larvae moulted in to the 4th instar on the 8th day. The influence of 1st instar duration on 3rd instar larval duration was insignificant (p=0.686947) (Table 20).

No mortality was observed and majority of those larvae (44.44%), which had spent 4 days in the 1st instar larval stage, completed their 4th instar on the 8th day, if the 1st larval period had lasted for 4 days. No significant influence of 1st instar on the 4th instar was observed (p=0. 050889) (Table 21).

44.44% of those pre-pupae that had taken 4 days to complete the 1st instar moulted into pupae on the 5th day. The effect of 1st instar duration on the pre-pupal period was insignificant (p=0. 939773) (Table 22).

Maximum number (33.33%) of those pupae that had consumed 4 days in the 1st instar moulted into adult on the 7th day. No significant influence of 1st instar larval period on pupal duration was observed (p=0.429875) (Table 23).

Influence of 2nd instar duration on the duration of subsequent instars

66.67% of the larvae, which spent 4 days in the 2nd instar, completed the 3rd instar on 5th day. The influence of duration of 2nd instar on 3rd instar larval period was insignificant (p=0. 251786) (Table 24).

When 4 days were spent in the 2nd instar larval stage, 66.67 % of the 4th instar larvae moulted into pre-pupae on 6th day. No significant influence of the 2nd instar larval period on 4th instar larval duration was found (p=0.635110) (Table 25).

58.3% of the pre-pupae, which spent 8 days in the 2nd instar, moulted in to pupae on the 5th day. The effect of 2nd instar larval duration on the pre-pupal period was insignificant (p= 0.108561) (Table 26).

41.18% of those pupae that had spent 6 days in the 2nd instar emerged as adult on the 9th day. No significant influence of 2nd instar larval duration on the emergence of the adult was observed (p=0.251079) (Table 27).

Influence of 3rd instar duration on the duration of subsequent instars

If the larvae spent 9 days in the 3rd instar, 71.43% of those 4th instar larvae moulted in to the pre-pupae on the 6th day. The influence of time spent in the 3rd instar on 4th instar larval duration was insignificant (p=0.683321) (Table 28).

57.14% of those larvae that spent 9 days in the 3rd instar completed pre-pupal stage in 6 days. The moulting of the pre-pupae was not influenced by the time the larvae had spent in the 3rd instar (p= 0.641163) (Table 29).

35.71% of those pupae that had spent 5 days in the 3rd instar moulted into adults on the 11th day. No significant influence of 3rd instar on the emergence of the adult was observed (p=0.636292) (Table 30).

Influence of 4th instar duration on the duration of subsequent instars

57.14% of those pre-pupae, that had spent 7 days in the 4th instar, moulted in to pupae on the 5th day. The effect of 4th instar duration on the duration of the pre-pupae was insignificant (p= 0.722223) (Table 31). Those pupae (50%) that had spent 5 days in the 4th instar moulted into adults on 11th day. No significant influence of 4th instar duration on the emergence of the adult was observed (p=0.074576) (Table 32).

Influence of pre-pupal duration on duration of pupae

71.43% of those adults which had a pre-pupal duration of 5 days emerged from the pupae on 11th day. The time spent in the pre-pupal stage significantly influenced the pupal duration (p=0.019657) (Table 33).

Influence of different developmental stages on subsequent instars at 32.5^0C and L:D 12:12

Influence of incubation period on duration of subsequent instars

88.46% of the 1st instar larvae which had spent 2 days in the incubation period moulted in to the 2nd instar on the 3rd day, while 75% of the larvae which had an incubation period of 3 days completed 1st instar on the 3rd day. The days spent in incubation did not influence the duration of 1st instar larvae (p= 0.426270) (Table 34).

35.29% of the 2nd instar larvae, which had spent 2 days in the incubation period moulted into the 3rd instar on the 6th day and 50% of those larvae, which had an incubation period of 3 days, also completed the 2nd instar on the 6th day. The 2nd instar larval period was not influenced by the incubation period (p= 0.847996) (Table 35).

4.17% of the larvae died and 68.75% of the 3rd instar larvae moulted in to the 4th instar on 5th day when the larvae had an incubation period of 2 days. 62.50% of the larvae completed the 3rd instar on the 5th day if they had an incubation period of 3 days. The duration of the 3rd instar was independent of the time spent in the incubation period (p=0.605499) (Table 36).

60.87% of the 4th instar larvae, which had incubated for 2 days, moulted in to the pre-pupae on the 6th day. The days spent in the incubation did not influence the 4th instar larval duration (p=0.826331) (Table 37).

It is noted that time spent in incubation did influence the pre-pupal period. 76.09% of pre-pupae, which had an incubation period of 2 days, moulted in to the pupal stage in 3 days. But 50% of the prepupae, which had spent 3 days in the incubation period, moulted in to pupae on the 4th day (p= 0.000954) (Table 38).

89.13% and 75% of those adults, which had spent 2 and 3 days respectively in the incubati0on period emerged from the pupae on the 6th day. The duration of pupal period was not influenced by the time spent in incubation period (p= 0.272129) (Table 39).

Influence of 1st instar larval period on duration of subsequent instars

42.31% of the 2nd instar larvae, which had spent 3 days in the1st instar, moulted into the 3rd instar on the 6th day. Mortality of the 2nd instar larvae, which had expended 4 days in the 1st instar, was 14.29%. The days spent in the 1st instar did not influence the 2nd instar larval duration (p=0.050474) (Table 40).

4% mortality was observed in those 3rd instar larvae, which had a 1st instar larval duration of 3 days. But no 3rd instar larvae, which had a 1st instar larval duration of 4 days died. 70% of those larvae which had 1st instar duration of 3 days completed the 3rd instar on the 5th day. The time spent in the 3rd instar was not influenced by the duration of the 1st instar (p=0.365879) (Table 41).

60.42 % of the 4th instar larvae emerged on the 6th day if the larvae used up 3 days in the 1st instar stage. No impact of the 1st instar duration was observed on the 4th instar larval period (p=0.609147) (Table 42).

68.7% of the pre-pupae that had used up 3 days in the 1st instar moulted in to pupae on 3rd day. The pre-pupal duration was significantly influenced by the 1st instar larval duration (p=0.005058) (Table 43).

87.50% of the pupae, which had a 1st instar larval duration of 3 days moulted into adults after 6 days. The days spent in the 1st instar did not influence the pupal period (p=0. 774518) (Table 44).

Influence of 2nd instar larval period on the subsequent larval periods

All those 3rd instar larvae, which had expended 8 days in the 2nd instar, moulted in to the 4th instar on the 5th day. But out of those larvae, which had spent 7 days in the 2nd instar, 80% completed the 3rd instar on the 5th day. 16.67 % and 14.29 % mortality of the 3rd instar was observed when the 2nd instar larval period was 4 and 5 days respectively. The p value was found to be insignificant (p=0.137071) (Table 45).

68.18% of the 4th instar larvae moulted into pre-pupae on the 6th day when it had consumed 6 days in the 2nd instar. No significant influence of the 2nd instar larval duration on 4th instar duration was observed (p=0.363561) (Table 46).

63.64% of pre-pupae that had spent 6 days in the 2nd instar moulted into pupae on the 3rd day (Table 47)

All the pupae which had spent 4 or 8 days in the 2nd instar, moulted into adult on the 6th day. p value was found to be insignificant (p= 0.514544) (Table 48).

Influence of 3rd instar larval period on the subsequent larval periods

66.67% of the 4th instar larvae moulted into pupae on the 6th day if it had expended 7 days at the 3rd instar stage. p value was found to be insignificant (p=0.544711) (Table 49).

All the pre-pupae that had a 3rd instar duration of 10 days died. p value was found to be insignificant (p=0.580193) (Table 50).

100% of the pupae emerged as adults on the 6th day if they had a 3rd instar larval duration of 7 days. But if the 3rd larval duration was 5 days, 89.47% of those adults emerged from the pupae on 6th day. No significant

difference in the influence of the time spent in the 3rd instar on the pupal duration was observed (p=0.341592) (Table 51).

Influence of 4th instar duration on the pre-pupal and pupal periods

83.33% of the pre-pupae moulted into pupae in 3 days if it had spent 7 days in the 4th instar. If the 4th instar larval duration was 5 days, 50% of the pre-pupae moulted in to pupae on the 3rd day. P value was not significant (p=0.091772) (Table 52).

When the 4th instar larval duration was 6 days, 93.75% of pupae emerged as adults on the 6th day. While 80% of those pupae moulted into adult on 6th day if it had spent 5 days in the 4th instar. No significant difference in the influence of 4th instar on the duration of the pupae was observed (p= 0.196136) (Table 53).

Influence of duration of pre-pupal period on pupal period

100% of the pupae, which had a pre-pupal period of 2 days, underwent eclosion on the 5th day, and if the pupae had a pre-pupal duration of 4 or 5 days, all the pupae moulted into adult on the 6th day. The days spent in the pre-pupal period influenced the duration of the pupal period (p=0.001326) (Table 54).

Discussion

Statistical analysis was conducted to find whether the duration of incubation period or the different instars had any influence on the duration of the following instars. It was noted that time spent in the incubation period and some instars did influence the duration of the subsequent instars. But in majority of the cases no significant difference in the influence of the incubation period or the instar duration on the duration of subsequent developmental stages was observed.

At 25°C,L: D 24:00, when incubation period was 3 days, the larval period was extended and 5.26% of the 1st instar larvae emerged on 29th day. Similarly at 25°C L: D 12:12, when incubation period was 2 days, 3.03% of the 1st instar larvae emerged on 30th day and the larval period was spread out and prolonged to 21 days at 25 L: D 00:24. Whereas at 30°C L: D 24:0, the 1st instar larval period was completed within 7 days. At 30°C L: D 12:12, all the 1st instar larvae moulted in to the 2nd instar within 6 days. This indicates that lower temperatures do prolong larval development, while it is faster at higher temperatures.

Among the different photoperiods at 25°C, total larval developmental period was longer at L: D 24:00 when the incubation period was 3 days. Whereas under L: D 00:24 photoperiod, the total larval developmental period was comparatively shorter. In contrast to what was observed at 25°C under various photoperiods, the larval development was completed within 8 days irrespective of the length of the incubation period at 30°C under different photoperiods.

The longer duration for completing larval development and higher mortality of all the instars at all photoperiods at 25^0C when the incubation period was prolonged shows that, the longer the time spent in the incubation period lesser the chance of the following instars surviving, may be due to some physiological effect. As a general rule, if incubation period is short, mortality is lower and growth rate faster in the latter instars.

At 25°C, total larval developmental period was almost equal under L: D 24:00 and 12:12 photoperiods, but was prolonged when compared to the total developmental period at 00:24 photoperiod at 25°C and under all photoperiods at 30°C.

At both the temperatures (25°C and 30°C), L: D 00:24 was the photoperiod under which total larval development period was the shortest, whereas under L: D 24:00, the duration of the total development cycle was the longest.

No pre-pupae moulted into pupae at any of the photoperiods tested at 25°C. Among the various photoperiods at 30°C, the larval developmental period was comparatively shorter at L: D 12:12 when incubation period was 3 days. This observation attains significance in the context of the prevailing photoperiod in Kerala.

It was observed that the extended duration for all individuals to complete an instar at certain temperatures does influence the fate of the individuals in the subsequent instars; as was observed at 25^0C at all photoperiods tested, where no individuals reached the pre-pupal stage.

The duration of 1^{st} instar was found to have significant influence on the duration of the 2^{nd} instar larvae of *N. rufipes*. Similarly duration of the pre-pupae was also significantly influenced by the pupal duration at 30°C and L: D 00:24 photoperiod regime.

At 32.5°C, L: D 12:12, it was observed that only the duration of pre-pupae was influenced by the time spent in incubation, 1^{st} instar and 4^{th} instar. All the other instars were not in any way affected by the time spent in incubation period and other instars.

In conclusion the results indicate that delay in completion of an instar does not necessarily mean that the subsequent instar duration will be affected. Time spent in each instar is independent of the duration of other instars.

CHAPTER IV

LONGEVITY

Effect of Temperature and Photoperiod on Longevity

Results

Longevity of male and female *N. rufipes* at a temperature of 25°C, relative humidity of 60% and different photoperiods of 12:12 (table 63, fig. 9), 24:0 (table 65, fig. 11) and 0:24 (table 64, fig. 10) were found to vary. The males were found to live for a mean of 54.49 days (range 15-90 days) and females for 62.49 days (range 24-90 days) at a photoperiod of 12:12 and a temperature of 25°C and RH of 60%. Adult males and females lived for a mean of 46.77 days (range 11-77 days) and 55.64 days (range 21-82 days) respectively at 24:0, whereas the mean longevity of male was a mean of 57.53 days (range 17-90 days) and that of female 66.47 days (range 27-101 days) at 25°C; 0:24. Mean longevity of adult male at 30°C and 60% RH and a photoperiod of 12:12 (table 59,fig. 5), 24:0 (table 61, fig. 7) and 0:24 (table 60, fig. 6), was a mean of 70 days (range 22-109 days), 58.61 days (range 20-90 days) and 80.04 days (range 31-130 days) respectively while that of the female was 79.16 days (range 38-119 days), 67.79 days (range 31-101 days) and 93.63 days (range 44-152 days) respectively.

At 32.5°C, L: D 12:12, the longevity of *N. rufipes* male was a mean of 83.34 days (range 26-134 days), whereas that of female was 95.59 days (range 41-153 days). The longevity of male and female was found to be significantly different at 32.5°C (p= 0.020667) (table 66, fig. 12).

The male *N. rufipes* lived for a mean of 41.60 days (range 7-72 days) and female for 48.18 days (range 7-73 days) at 35°C, L:D 12:12. Longevity of adult male and female was significantly different (p=0.043139) (table 62, fig. 8).

Significant difference in mean longevity of adult males (table 55 and 56, fig. 2) and females (table 57 and 58, fig. 3) at different temperatures and photoperiods were observed. A comparison of the mean longevity of adult male and female at different temperatures and photoperiods is given in fig. 4.

Discussion

Comparison between the longevity of *N. rufipes* male and female indicated that the adult beetles lived longer at 32.5°C and L: D 12:12 than at any other temperatures and photoperiods tested. It was observed that the male and female *N. rufipes* had maximum longevity at 32.5°C (male 134 days; female 153 days), followed by 30°C (male 109 days; female 119 days), 25°C (male 90 days; female 90 days) and 35°C (male 72 days; female 73 days).

Though the increase in temperature up to 32.5°C increased the longevity, it declined at 35°C. Studies on *Dermestes frischii* have also shown that longevity of beetles is higher at 30^0C than at 35^0C (Amos and Morley, 1971). However, Azab *et al.*, (1973 a) had observed that adult males and females of *D. maculatus* lived for up to 189 and 178 days respectively at 21.5^0C. In the present study, at all temperatures and photoperiods tested, *N. rufipes* females lived longer than males and the maximum longevity for both males and females was obtained at 32.5^0C, L: D 12:12, than at a lower temperature of 25^0C. While at 35^0C, L: D 12:12, the mean longevity of *N. rufipes* males and females was 72 days and 73 days respectively, in the case of *D. maculatus* it was only 49.1 and 51.9 days respectively (Azab et al., 1973 b). This increased longevity at higher temperatures in *N. rufipes* may help it in outcompeting *D. maculatus* in the field when the summer temperatures peak.

At 23-25^0C longevity of male and female *D. maculatus* was 70 and 76 days respectively (Shahhosseini, 1980), while at this temperature adult male

and female of *N. rufi*pes lived for 90 days. Adults of *D. lardarius* lived for only 36 days at 30^0C, while adults of *N. rufipes* lived for 109 (males) and 119 (females) days (Jacob and Fleming, 1980a). These results emphasize the temperature tolerance of *N. rufipes* when compared to other species of *Dermestes* reared on a diet of fish.

The reason for the high infestation of *N. rufipes* during the summer, when temperature in the stocking shed is in the range 30-33°C, can be explained by the results of the present study, where the ideal temperature for growth of *N. rufipes* was observed to be 32.5°C. The extended longevity in the laboratory at 32.5°C correlates with its observed incidence in the field. A steep decline in the population of the beetle during rainy season when the temperature hardly reaches 25°C inside the stocking shed could also be explained on the basis of the results of the present study.

During the monsoon, when dried fish were not stocked in the stocking sheds, a residual population of the pest was seen in the fish refuse left back in the sheds. This may migrate in to the fresh stock of fish when dried fish are again stocked here and start a new infestation.

At temperatures (25°C and 30°C) and photoperiods (L: D 12:12, 24:0, 0:24), highest longevity was observed when they were reared in full darkness (L:D 00:24). *N. rufipes* was found to be photonegative and the observations in the field have also shown that *N. rufipes* preferred dark places. Most of the stocking sheds along the coastal belt of Kerala do not have any lighting, which is the ideal environment for the pest and it may help in better propagation of the beetle pest. Increased longevity ultimately leads to excessive reproduction as the beetle continues to lay eggs till death. Increased longevity also means persistent pest attack on stored dried fish in the stocking shed.

Prolific pest infestation was observed in the Puthiappa, Tanur, Tirur and Parappanangadi coastal areas where the stocking sheds were made of pleated palm leaves with little or no ventilation. As a result of this, the temperature as well as humidity increases to facilitate increased pest attack which affects the quality and quantity of the product eventually leading to tremendous economic loss to producers. While in Ponnani, Kasargod, Alleppey, Kollam and Thiruvananthapuram where many of the stocking sheds were made of concrete structures with moderate aeration and ventilation and experiences a 12:12 photoperiod as it is in the field, the population density was found to be moderate and obviously lower than what was observed in a stocking center in complete darkness (0:24).

In Alleppey district where there was a practice of stocking the dried fish in the open sand on the seashore, the pest attack was found to be less in comparison with those piled up inside the stocking shed. Unfavorable temperature and photoperiod were considered to be the limiting factors.

CHAPTER V

REPRODUCTION

Courtship and Mating behaviour

Results

The first contact between male and female was observed to be purely by chance. Male perceives the female when they are about 5 mm apart. The male then turns towards the female. The moment the male and the female comes in contact, the male hurriedly tried to mount her. After mounting, the male tapped the anterior part of the abdomen of the female with his antennae and rubbed her with his forelegs in the prothoracic region. The female was observed to be either motionless or to run very fast with the male on top of her. If the female did not move, the male moved down and aligned the tip of last segment of its abdomen with that of the female all the while tapping her with his antennae, rubbing the thoracic region with forelegs and the middle legs and scratching the thorax with the mandible. The male lowered the tip of his abdomen and extruded his copulatory organ. The aedeagus moved down and extended forward for 2-3 millimeters until it contacted the tip of the female's abdomen. Receptive female raised the tip of her abdomen and slightly extruded her ovipositor to allow intromission. When the male achieved intromission, he stopped moving and gradually ceased rubbing his foreleg and middle leg on the prothorax and antennae tapping, whereas the scratching with the mandible on the thorax of the female continued. Intromission occurred only when the female stopped moving. Both male and female continuously move the antennae during the entire act of mating. Only the hind legs of the male touched the substratum during copulation. Male remained mounted for 30 seconds to 7 minutes and copulated with the female several times. The time gap between successive copulation was found to vary (at the beginning 3-4 seconds which gradually increased up to 10-12 seconds towards the end).

If the female is unreceptive it dislodges the male. The male follows her and tries to mount her again. Repeated rejections resulted in the male giving up the effort. Mandible cleaning by both sexes was observed throughout the copulatory act. Mate guarding has been observed in *N. rufipes*. Male remained on top of the female after withdrawing his copulatory organ or sometimes dismounts the female and stays immediately adjacent to and in physical contact with her for 3 to 7 seconds. When guarding, the male frequently strokes the body of the female with his antennae and maxillary palps. The female remained relatively quiescent during the majority of the guarding period. Whenever she moved away from the male, he made rapid searching movements and followed the female until he regained contact with her. It was also observed that when the pairs were left undisturbed, the male goes straight from guarding into a new round of courtship and mating. Guarding males behaved aggressively toward members of their own sex and attacks intruding males.

Discussion

Mate guarding observed in the present study has been observed in other insects also. In the oriental beetle, mating and copulation occurred without an obvious complex courtship, but observations of post mating behaviours suggested that mate guarding occurs (Facundo, *et al*., 1999).

Most positions in insect mating appear to be derived from a situation in which the female climbs on the back of the male (Cade, 1985), but in the present study males are above females in the so-called male superior position as is seen in case of female crane flies, *Tipula oleracea* (Diptera:Tipulidae) (Stich,1963).

Effect of Temperature and Photoperiodicity on Fecundity

Results

25^0C, L: D 12:12

The mean number of eggs laid by freshly emerged mated female /day was 0.585 eggs (1-11 eggs) after multiple mating. The total fecundity due to multiple mating was found to be a mean of 39.55 eggs, while it was a mean of 22 eggs for double mating which was significantly different (p= 1.38E-06) (fig. 30). The rate of oviposition peaked between 40^{th} and 50^{th} day during multiple mating (fig. 37). In the case of double mating, 1^{st} mating produced a mean of 9.05 eggs and 2^{nd} mating produced a mean of 12.95 eggs and it was significantly different (p= 0.000123) (fig. 29). The oviposition period of freshly emerged multiple mated females was a mean of 66.2 days, while the pre-oviposition period was a mean of 2.25 days. The ovipositing females died one day after egg laying stopped.

25^0C L: D 00:24

Freshly emerged mated female *N. rufipes* laid a mean of 0.682 eggs / day (1-16 eggs) after multiple mating. The total number of eggs laid by adult female was a mean of 52.75 eggs after multiple mating, while it was a mean of 34.6 eggs for double mating and it was significantly different (p= 2.33E-09) (fig. 32). The oviposition rate was highest between 40^{th} and 50^{th} day after multiple mating (fig. 36). During double mating, 1^{st} mating produced a mean of 12.85 eggs and the 2^{nd} mating produced a mean of 21.75 eggs and the fecundity rate was significantly different (p= 4.25E-08) (fig. 31). The pre-oviposition and oviposition period of freshly emerged multiply mated females were a mean of 1.35 days and 70.4 days respectively. The ovipositing females died 2 days after egg laying stopped.

25^{0}C, L: D 24:00

A mean of 0.45 eggs were laid by freshly emerged mated female/ day (range 1-6 eggs) after multiple mating. The fecundity due to multiple mating was found to be a mean of 25.4 eggs, while it was a mean of 17.5 eggs for double mating which was significantly different (p= 7.19E-05) (fig. 34). The maximum number of eggs was laid between 30th and 40th day after multiple mating (fig 35). A mean of 7.3 and 10.2 eggs were laid after the 1st and 2nd mating respectively and it was significantly different (p= 0.000151) (fig. 33). The oviposition period of freshly emerged and multiply mated females was a mean of 56.45 days, while the pre-oviposition period was a mean of 3 days. The ovipositing females died one day after egg laying stopped.

30°C, L: D 12:12

Adult female laid a mean of 1.97 eggs /day (0-26) after multiple mating. Adult female laid a mean total of 122.15 eggs due to multiple mating and 71 eggs for double mating, which was significantly different (p=5.96E-08) (fig. 39).The rate of oviposition peaked between 30th and 40th day for the multiple mating (fig. 44). In the case of double mating, 1st mating produced a mean of 28.15 eggs and the second mating produced a mean of 42.85 eggs and it was significantly different (p=0.010268) (fig 38). The oviposition and pre-oviposition period of freshly emerged multiple mated females was a mean of 62.05 days and 1.2 days respectively. The ovipositing females died 2 days after egg laying stopped.

30°C, L: D 00:24

The mean number of eggs laid /day/female was 1.60 (range 1-39) after multiple mating. The total fecundity due to multiple mating was found to be a mean of 158.1 eggs, while it was a mean of 90.65 eggs for double mating which was significantly different (p=1.53537E-09) (fig. 41).The

oviposition rate peaked between 60th and 70th day for multiple mating (fig. 45). A mean of 35.75 and 54.9 eggs were laid after 1st and 2nd mating respectively after double mating and it was significantly different (p=0.0003193) (fig. 40). The multiple mated freshly emerged females laid eggs for a mean of 100.45 days. The pre-oviposition period of freshly emerged multiple adult females was a mean of 1.2 days. The ovipositing females died 3 days after egg laying ceased.

30°C, L: D 24:00

N. rufipes laid a mean of 1.53 eggs/day (range 1-21 eggs) during multiple mating. The total fecundity due to multiple mating was found to be a mean of 99.1 eggs, while it was a mean of 53.4 eggs for double mating which was significantly different (p=7.64E-11) (fig. 43). The oviposition rate was highest between 40th and 50th day during multiple mating (fig. 46). In the case of double mating, 1st mating produced a mean of 21.85 eggs and the second mating produced a mean of 31.55 eggs and it was significantly different (p=0.000338) (fig. 42). The oviposition period of multiple mated freshly emerged females was a mean of 62.4 days. The pre-oviposition period of freshly emerged multiple adult females was a mean of 2.05 days. The ovipositing females died one day after egg laying stopped.

32.5 ^{0}C, L: D 12:12

The mean number of eggs laid/day after multiple mating in the case of newly emerged female *N. rufipes* was a mean of 2 eggs (1-63); where as that of 15-day old adults was 1.63 eggs. The fecundity due to multiple mating of newly emerged adult was found to be a mean of 227.7 eggs and the oviposition period lasted for a mean of 115.05 days. Fecundity was a mean of 148.4 eggs for double mating which was significantly different. (p=1.07 E-13) (Table 80, fig. 28). Multiple mated 15-day old females laid a mean of 206.65

eggs, whereas double mated 15-day old females laid a mean of 123.21 eggs (table 79, fig. 27). Multiple mated 15-day old females laid eggs for a mean of 101 days. In the case of double mating of the newly emerged adults, 1st mating produced a mean of 60.35 eggs and the 2^{nd} mating produced a mean of 88.05 eggs and it was significantly different. (p=2.6E-08) (fig. 25). The fecundity due to 1^{st} and 2^{nd} mating of the 15-day old adults was found to be 42.45 eggs and 80.76 eggs respectively. The difference in fecundity was significant (p=0.43) (fig. 26). The oviposition rate peaked between 60^{th} and 80^{th} day for the multiple mating in the case of freshly emerged adults (fig. 47), while it was on the 50th day for multiple mating in the case of 15-day old adults (fig. 48). The pre-oviposition period for 15-day old multiple mated female was a mean of 1.15 days, while that of adult females mated just after emergence was a mean of 1.2 days. Freshly mated and 15-day old mated females died 3 days after egg laying ceased.

Comparison of fecundity at different temperatures (25 and 30^0C)and photoperiods (L:D, 12:12, 00:24, 24:00)

The mean number of eggs laid after double mating at 25 and 30^0C, L:D 0:24 was significantly different (p=0) (Table 67, fig. 13). Significant difference was also observed in mean number of eggs laid after multiple mating at the same temperatures and photoperiod combination (p=0) (Table 68, fig. 14).

The mean number of eggs laid after double mating at 25 and 30^0C, L: D 24:0 was significantly different (p=0) (Table 69, fig. 15). Significant difference was also observed in mean number of eggs laid after multiple mating at the same temperatures and photoperiod combination (p=0) (Table 70, fig. 16).

At a photoperiod of L: D 12:12, and 25 and 30^0C, the mean number of eggs laid after double mating was significantly different (p= 5.57E-11) (Table 71, fig. 17). Significant difference was also observed after multiple mating at the same temperature photoperiod combination (p=5.37E-16) (Table 72, fig. 18).

Significant difference in the mean number of eggs laid after double mating at 25^0C and the three-photoperiod combinations was observed (p=1.16E-15) (Table 73, fig 19). The mean fecundity after multiple mating at this temperature and three photoperiod combinations was also significantly different (p= 2.02E-11) (Table 74, fig. 20).

Significant difference in the mean number of eggs laid after double mating at 30^0C and the three photoperiod combinations was noted (p=1.8E-05) (Table 75, fig. 21). The difference in mean fecundity after multiple mating at this temperature and three photoperiod combinations was also significant (p=2.28E-10) (Table 76, fig. 22).

The mean fecundity after double mating at the three temperatures (25, 30 and 32.5) at L:D 12:12 was significantly different (p=1.56E-23) (Table 77, fig. 23), while the difference in fecundity was highly significant (p=0) during multiple mating at the above three temperatures and photoperiod (Table 78, fig. 24).

Discussion

Studies on the fecundity are essential to understand the population dynamics of a pest. With the exception of studies done on *N. rufipes* by Simmons and Ellington (1925) on a diet of stale bacon, no work has been carried out on this insect as a pest of dried fish. This is the first study of its kind to determine the fecundity of this pest on a diet of dried fish and also to understand the effect of temperature and photoperiod on fecundity. The

significant difference in fecundity when results of different temperature and photoperiod combination were compared emphasizes the influence of temperature and photoperiod on egg laying in *N. rufipes*.

In the present study, the fecundity of *N. rufipes* fed on dried fish was a mean of 227.7 eggs at 32.5^0C and L: D 12:12, which was much higher than that observed by Simmons and Ellington (1925) (a mean of 137 eggs) when *N.rufipes* was fed on stale bacon- (temperature not mentioned). This difference may have been due to the difference in diet and the environmental conditions. Osuji (1975 d) had noted a mean fecundity of 200 eggs, for *Dermestes maculatus* reared on dried fish, which is still less than that observed in the present study with *N. rufipes*.

When a photonegative insect is studied for its pest status, it is imperative to study the effect of photoperiods on biology, longevity, food consumption etc. Rakowski (1988) observed that 80% of *D. maculatus*, a pest of dried fish chose the dark rather than the illuminated part of its surroundings and proved that the intensity of light played an important role in its behaviour.

The effect of photoperiod and temperature on fecundity has been recorded in other insect species also. *Megalurothrips sjostedti* laid 7.1 eggs at 25°C, L: D 12:12 photoperiod regime (Ekesi et al., 1999). Over a 24h period, at 25°C, L: D 12:12 photoperiod combination, *Frankiniella occidentalis* also laid few eggs (4 eggs) (Whittaker and Kirk, 2004), whereas the present study has indicated that *N. rufipes* at similar temperature- photoperiod combination laid a mean of 39.55 eggs.

When *P. xylostella* females were paired with virgin males, 78% of females mated once and only 19.3% females mated twice. The rates of mating females were reduced significantly after their first mating (Wang et al., 2005). But this study differs from the above studies in that *N. rufipes* male and

female continue to mate till death. *N.rufipes* female produced a mean of 9.05 eggs during first mating at 25°C, L: D 12:12, whereas the first mating of *Plutella xylostella* female produced significantly higher numbers of eggs (127.87eggs) at almost similar temperature-photoperiod combination (Wang *et al*., 20052nd mating of the female *P. xylostella* produced 143.18 eggs at 25°C; 75% RH and under L16:D8 photoperiod regime (Wang et al., 2005), whereas the second mating of the *N. rufipes* gave only 12.95 eggs, which shows that *N. rufipes* is not adapted to lay eggs at low temperatures.

At 25°C and a photoperiod of L: D 12:12, 2nd mating of *N. rufipes* produced more eggs (12.95) than the 1st mating (9.05) after the double mating. At the same temperature but at a photoperiod of L: D 00:24, significant increase in fecundity was observed where 1st and 2nd mating of freshly emerged females produced a mean of 12.85 and 21.75 eggs respectively during double mating. 25°C and L: D 24:00 combination significantly reduced the oviposition potential of the adult female where the 1st and 2nd mating produced only a mean of 7.3 and 10.2 eggs respectively after double mating. A photoperiod of 00:24 was found to give the highest fecundity (34.6 eggs) in newly emerged adult female at 25°C than the other photoperiods tested such as L: D 12:12 (22 eggs) and 24:00 (17.5 eggs). These results emphasize the photonegative nature of the insect and its better adaptation to darkness.

Coombs (1979) observed that at 25^0 C and 65% R.H., *Dermestes haemorroidalis* and *D. peruvianus* laid 170.2 eggs and 3.9 eggs respectively, while Jacob and Fleming (1984 a) recorded an average of 26.81 eggs in *Dermestes lardarius* at 25°C, 65% R.H. But in the present study at the same temperature and humidity, *N.rufipes* laid a mean of 39.55 eggs. Although the fecundity of *N. rufipes* compares favorably with that of *D. lardarius* and *D.*

peruvianus it is considerably less than the fecundity realized in the case of *D. haemorroidalis*.

Jacob and Fleming (1984a) reported that in *D. lardarius* there were two oviposition periods consisting of a short first period and a longer second period of oviposition at various temperature and humidity. It was also observed by the same authors that females at 25^0C started to lay eggs 14-35 days after emergence. But in the present study, *N. rufipes* oviposit 2-3 days after mating and continue to lay eggs until 3-4 days before death and had just one oviposition period at all temperatures and photoperiods tested.

The oviposition period was longest at 32.5^0C and decreased as temperature decreased in the present study. But in *D. maculatus* Azab et al. (1973 c) had observed that oviposition period was extended as temperature decreased from 35^0C to 21^0C and peak number of eggs laid occured at 27^0C. In the case of *N. rufipes* highest number of eggs laid was at 32.5^0C.

Majority of female *D. lardarius* ceased egg laying above 25°C and 12:12 photoperiod regime (Jacob and Fleming, 1984a), where as all *N. rufipes* females laid more eggs above this temperature at L: D 12:12 photoperiod (a mean of 122.15 eggs at 30°C; L: D 12:12 photoperiod combination)

Frankiniella occidentalis laid 2.5 eggs at 25°C and L: D 00:24 photoperiod during multiple mating (Whittaker and Kirk, 2004) in contrast to a mean of 52.75 eggs laid by *N. rufipes* in this study. 5.5 eggs were laid at 25°C, L: D 24:00 photoperiod combination by *F. occidentalis* (Whittaker and Kirk, 2004), which was considerably less when compared to the fecundity of 25.4 eggs in *N. rufipes* observed in this study.

At 30°C, R.H. 60% and L: D 12:12 photoperiod, *N.rufipes* laid a mean of 122.15 eggs, while the fecundity of *D. peruvianus* at 30°C; 60% r.h. was

29.4 eggs (Coombs, 1979) and was contrary to the result observed with *D. lardarius* by Jacob and Flemming (1984a) where no eggs were laid at 30°C.

N. rufipes laid a mean of 158.1 eggs at 30°C, L: D 00:24, whereas *M. sjostedti* laid only a mean of 6.3 eggs at (Ekesi *et al*., 1999) similar temperature-photoperiod combination. *M. sjostedti* ceased egg laying at 30°C, L: D 24:00 photoperiod regime (Ekesi *et al*., 1999), whereas *N.rufipes* laid 99.1 eggs at similar temperature and photoperiod. This indicates the better adaptation of *N. rufipes* to this temperature –photoperiod combination.

At all the three temperatures (25°C, 30°C and 32.5°C) and photoperiod combinations (12:12, 00:24 and 24:00) in double mating, second mating produced more eggs than after first mating in *N. rufipes*, which may be an indication that females need to be more mature to realize their maximum biotic potential. This result is strengthened by the observation that in all multiple mating cases also the oviposition rate peaked later in the female oviposition period.

An increase in the temperature up to 30°C resulted in an increased fecundity at different photoperiod combinations in comparison to the fecundity at 25°C and all the photoperiod combinations tested. At 30°C and a photoperiod regime of L: D 12:12, adult female laid 122.15 eggs after multiple mating, whereas only 99.1 eggs were produced by multiple mated females at L: D 24:00 photoperiod combination. A significant increase in fecundity (158.1 eggs) was observed at 30°C and L: D 00:24 photoperiod regime and this combination was found to be the most successful one in producing maximum number of eggs in comparison to the other two photoperiods tested at 30°C.

At 30°C and L: D 00:24 photoperiod combination, mated females laid more eggs both in double mating (90.65 eggs) and multiple mating (158.1

eggs) in comparison to that laid during double mating (34.6 eggs) and multiple mating (52.75 eggs) at 25°C in the same photoperiod. At 30°C and L: D 12:12, mated females produced more eggs both in double mating (71 eggs) and multiple mating (122.15 eggs) in comparison to that produced during double mating (22 eggs) and multiple mating (39.55 eggs) at 25°C L:D 12:12.

The results of this study shows that multiple mating may be the normal mode of mating in *N. rufipes* as the insects lays more eggs when multiply mated than when mated singly at all temperature-photoperiod combinations. Multiple mating is prevalent in insects although females store sperm in spermatheca, and a single mating is often sufficient to fertilize all of a females eggs (Wigglesworth, 1965). Repeated mating did not affect the fecundity or viability of *Trogoderma versicolor* females, however, since singly and multiply mated females produced roughly equal numbers of progeny (Norris, 1936). But it was observed in the present study that single mating allowed the females to lay eggs for an average of 54.5 days (at 32.5°C) only, and remating brought about further egg laying which lasted for a mean of 116.2 days at the same temperature and there was a significant difference in the fecundity of singly and multiply mated females.

Parker (1970, 1979) predicted that females must benefit reproductively from multiple matings. If not, then multiple mating behaviour will be selected against. Depending on the group of insects, the benefits of multiple mating may include the following: provision of additional spermatozoa if there is a high probability that initial matings were unsuccessful in transferring spermatozoa; avoidance of the physiological or morphological expense of sperm storage. In *N. rufipes* multiple mating may be normal mode of mating as it helps the female in laying more eggs than after a single mating. Azab *et al.*, (1973 c) has also observed that in *D. maculatus* to obtain

maximum number of eggs it was necessary to keep males and females together continuously. When the males were removed soon after first batch of eggs were laid, the total number of eggs obtained were reduced by about one-third.

The results of our studies are also in agreement with those of Sakaluk and Cade (1980, 1983) who observed that female house cricket *Acheta domesticus* mate more than once and that doubly mated females produced significantly more offspring than did females, which mated only once.

In *N. rufipes* single mating at 30°C resulted in the female ceasing egg laying after 30.1 days and further egg laying occurs only after another mating. This indicates that sperm storage ability may be limited in *N. rufipes*. It has also been noted that, in some insects females live sufficiently long and lay enough eggs that they simply run out of sperm. In these species the relative inexpense of repeated mating and the apparent availability of males have resulted in selection against additional sperm storage ability (Cade, 1985). Pyle and Gromoko (1978) studied repeated mating in *Drosophila melanogaster*. In this species, females which mated once produced 528 larvae/individual on average. Females, which were allowed to remate, however, produced 1053 offspring. Until 7 days singly mated females laid same number of eggs, but after that reduction in egg production was observed in single mated females. Marks (1976) also demonstrated that female red bollworms *Diparopsis castanea*, benefit from remating in that progeny production of singly mated females decreased by approximately 25% after 5 days of egg laying. By contrast, doubly mated females showed no drop in progeny production over a 10-day period. These observations in the above studies are in agreement with the results obtained in the present study on fecundity due to double and multiple mating in *N. rufipes*.

The results also indicate that 32.5°C was the preferred temperature at a photoperiod of L: D 12:12 to realize higher fecundity after both double and multiple mating when compared to the temperatures 25°C and 30°C. It was also observed that exposing the insects to a photoperiod of L: D 24:00 at 25°C and 30°C does decrease fecundity. The longest oviposition period was observed at 32.5^0C, L: D 12:12 and the shortest at 25^0C, 24:00.

It was also observed that in *N. rufipes* that at temperatures of 25, 30 and 32.5^0C, a photoperiod of 00:24 was the ideal one to realize the maximum fecundity. This again indicates that *N. rufipes* is adapted to darkness and will thrive in such conditions.

The stocking sheds throughout Kerala, were either covered with pleated palm leaves or tile roofed and covered all around by palm leaves or concrete walls without proper ventilation which ensures complete darkness throughout the day, an ideal condition for the better propagation of *N. rufipes*. Storing the dried fish in heaps or in sacks piled upon one another will further ensure complete darkness and the required temperature and humidity for realizing higher fecundity of the pest. Spreading the dried fish on the floor and packing it only when it is ready for transportation may help in preventing the proliferation of the pest, as the ideal conditions are not provided. Although it may not be economically feasible to store dried fish at below 25^0C, the results suggest that complete lighting of the storage sheds could lead to decrease in fecundity of the pest and hence contribute to its control.

It was also observed in *N. rufipes* that within a temperature regime, (either 25°C or 30°C) a photoperiod of 0:24 was the ideal one to realize the maximum fecundity. This again indicates that *N. rufipes* is adapted to darkness and will thrive in such conditions.

CHAPTER VI

FOOD CONSUMPTION

Food consumption at 30°C (L:D 24:0; 0:24,12:12), 32.5 and 35°C (L:D 12:12)

Results

The total amount of dried fish consumed by *N. rufipes* at 35 °C, L: D 12:12, to complete its developmental stages was a mean of 0.170257g, where as, it was 0.367342 g and 0.301161g at 32.5 and 30°C, L: D 12:12 respectively. The total food consumption at different temperatures was found to be significantly different (p=1.09E-08).

At 35 °C, L: D 12:12, 1st instar larva consumed a mean of 0.028235g of food, where as 2nd and 3rd instars consumed 0.057647g and 0.084375g respectively. Significant difference in food consumption between different instars was observed (p=7.56E-14) (Table 81). The significant difference in mean consumption between different instars is given in Table 82.

The 1st instar of *N.rufipes* consumed a mean of 0.035789g of dried fish and 2nd, 3rd and 4th instars consumed a mean of 0.0715g, 0.111053g, and 0.149g of dried fish respectively at 32.5 °C, L:D 12:12 (Table 83). Dried fish consumed by the various instars was significantly different (p=0) (Table 84).

At 30°C and photoperiods of L: D 00:24, 12:12 and 24:0, the total amount of dried fish consumed by all the four larval instars was a mean of 0.371111g, 0.301161g and 0.217182g respectively (p=1.09E-08) (Table 85)

The 1st instar consumed 0.018947g and the 2nd, 3rd and 4th instar consumed 0.042941g, 0.067647g, and 0.087647g respectively at 30 °C,L: D 24:00. The food consumption at 30 °C and 24:0 photoperiods by various instars was significantly different (p=1.68E-28) (Table 86). ANOVA between the different instars is given in Table 87.

At 30 °C and a photoperiod of L: D 0: 24**,** 1st, 2nd, 3rd and 4th instar of *N.rufipes* consumed a mean of 0.033889g, 0.075g, 0.114444g and 0.147778g of dried fish respectively. The food consumed by various instars at 30 °C; 0: 24-photoperiod regime was found to be significantly different (p=0) (Table 88). ANOVA between the different instars is given in Table 89.

1st, 2nd, 3rd and 4th instars of the beetle pest consumed 0.031875g, 0.061429g, 0.089286g and 0.118571g of food respectively at 30 °C, 12:12**.** Food consumed by the different instars was significantly different (p=3.03E-28) (Table 90). ANOVA between the different instars is given in Table 91.

Discussion

The highest food consumption (0.371111g) was observed at a temperature–photoperiod combination of 30°C and L: D 00:24. The photoperiod L: D 00:24 was the ideal photoperiod for better food consumption among all the photoperiods tested at 30 °C.

At 30 °C and L: D 12: 12, 24:00 and 00:24, there was an increase in food consumption from the 1st instar to the 4th instar.

The total amount of food consumed by all the instars at 32.5 °C (0.367342g) was observed to be greater than all other temperatures tested viz; 35 °C and □30°C at a photoperiod of L: D 12:12. But, at 35 °C only 0.170257g of dried fish was consumed which was the lowest. This indicates that at L: D 12:12, 32.5 °C is the ideal temperature for maximum food consumption. But this is lower than the total food consumption (0.37111g) at 30°C, 00:24.

At 35 °C,L: D 12:12, 3rd instar consumed more food (0.084375g) in comparison to the 2nd and 4th instars and the first instar consumed the least (0.028235g. The 4th instar at 32.5^0C, 12:12 consumed the highest quantity

(0.149g) of dried fish when compared to the other instars at all temperatures and photoperiods tested.

Maximum economic loss due to pest attack was observed in the field during the summer season when the temperature rises above 30°C. The present study on food consumption also indicates a significant increase in the feeding potential of *N. rufipes* at 30°C and 00:24-photoperiod regime. Although the mean weight of dried fish consumed by larvae of *N. rufipes* for completing its development cycle is low ((0.371111g) even at 30°C L:D 00:24 (the temperature-photoperiod combination at which the highest quantity of dried fish is consumed), thousands of larvae observed feeding in the field during peak pest infestation, may consume large quantities of dried fish and hence result in huge economic loss to the dry fish farmers. An increase in temperature above 32.5°C does bring about drastic reduction in food consumption (at 35°C food consumed is 0.170257 g). Lowest food consumption and highest larval mortality were observed at 35 °C. The results clearly indicate that the temperature played a vital role in the food consumption of *N. rufipes.*

In the field, low scale of attack by *N. rufipes* and hence minimum loss of dried fish was observed during the rainy season when the temperature was below 30 °C. But during the summer season, temperature increases above 30°C and may reach upto 39^0C along the coastal belt of Kerala. The present study revealed that pest infestation and hence loss of dried fish was at its peak during the summer season when the temperature was above 30°C and below 35°C. In addition, in many drying centers across Kerala, there is a practice of covering the piled up sacks and heaps of dried fish in the ground with plastic sheets, jute mats etc., which further help maintain darkness and a conducive temperature and humidity for better feeding and propagation of the pest.

Thus it was obvious that the climatic conditions that prevail all along the coastal belt of Kerala during summer season do enhance the proliferation of the

beetle pest, which ultimately results in the increased pest attack and ensuing massive economic loss.

CHAPTER VII

EFFECT OF LEAF EXTRACTS AND SPICE OILS

Effect of Leaf extracts and Spice Oils on the egg, larval and adult stages of *Necrobia rufipes*

Results

All results presented here are after 24 hrs. exposure to the leaf extracts and spice oils. No mortality at 48 and 72 hours was observed.

In this study, crude leaf extracts of *Glyricedia maculata*, *Pongamia pinnata*, *Chromolaena odoratum*, seed extracts of *Annona squamosa* and Neem extract (Nimbicidin) topically applied at different concentrations on the dry fish supplied as diet were ineffective on any stages of *N. rufipes* and no mortality was observed.

100% mortality of adult, egg, and all the larval instars were attained at 10%, and 5% of all the oils tested. But at 2%, 1%, 0.5% and 0.25% concentrations of all the oils tested, 100% mortality was achieved only in the case of 1st and 2nd instar larvae of *N. rufipes*.

At 2% and 1% concentrations of black pepper oil, 100% mortality of eggs, 1st and 2nd larval instars and adults were observed. 4th instar larvae registered the lowest mortality at all the concentrations tested (fig. 70).

1.291% of black pepper oil was required to kill 50% of the 3rd instar larvae of *N.rufipes*, while 2.843% and 0.260% of the oil killed 50% of 4th instar larvae and adults respectively (fig. 49, 50, 51).

LC50 value of crude Lemon Grass oil (CLGO) for 3rd instar was 0.009% and that of 4th instar and adult was 1.54% and 0.002% respectively (fig. 55, 56, 57). It was also observed that 100% mortality was recorded in the case of egg, 1st, 2nd, 3rd instar and adults at 5%, 2%, 1% and 0.5% concentrations of CLGO tested (fig. 72). The efficacy of black pepper oil was significantly different from crude Lemon Grass oil (p=0.008202) (Table 95).

Turmeric oil at concentrations of 2, 1, 0.5 and 0.25% killed 100% of eggs, 1st and 2nd larval instars of *N. rufipes* and the mortality of 3rd, 4th and adults was very low at 0.5 and 0.25% (fig. 75). LC50 value of Turmeric oil for 3rd and 4th instars and adults was 2.309%, 2.349% and 0.349% respectively (fig. 64, 65, 66). Crude Lemon Grass Oil (CLGO) was more effective than turmeric oil in causing larval mortality and the difference was significant (p=0.000501) (Table 95).

4th instar larvae were found to be more resistant than other developmental stages and adults to all concentrations (2%, 1%, 0.5% and 0.25%) of clove oil tested (fig. 71). When *N.rufipes* was fumigated with clove oil 50% of the 3rd and 4th instars and adult were killed at concentrations of 0.529%, 2.576% and 0.292% respectively (fig. 52, 53, 54). The CLGO was more effective in controlling the *N. rufipes* adult and various instars than clove oil (p=0.008202) (Table 95).

50% of the 3rd and 4th instar larvae and adults were killed at concentrations of 0.002%, 2.849% and 0.147% of Lemon Grass oil - A (LGO-A) respectively (fig. 58, 59, 60). Even at 5% concentration of LGO-A only 80% mortality of 4th instar larva was noted, while all the other stages had 100% mortality at all the concentrations tested (fig. 73).

At 1% concentration of Lemon Grass Oil –B (LGO-B), 100% mortality was observed in the case of all stages with the exceptions of 3rd and 4th instar (fig. 74). 0.313% of LGO-B killed 50% of the 3rd instar larvae and 3.397% and 0.232% of the same oil killed 50% of the 4th instar and adult respectively (fig. 61, 62, 63). There was no significant difference in the ability of LGO-A and LGO-B in controlling the beetle pest (p=0.994528) (Table 95).

LC50 value of Cinnamon oil for 3rd instar was 0.385% and that of 4th instar and adult was 2.0188% and 0.189% respectively (fig. 67, 68, 69). No significant difference in effect of LGO-A and Cinnamon oil on *N. rufipes* was

observed (p=0.647957) (Table 95). Cinnamon oil at 0.25% causes less than 40% mortality of 3rd and 4th instar larvae and adults of *N. rufipes* (fig. 76).

Significant differences in the effect of the different oils is given in table 92.

Discussion

In this study leaf extracts of *Glyricedia maculata, Pongamia pinnata, Chromolaena odoratum*, seed extracts of *Annona squamosa* and Neem extract (Nimbicidin) topically applied on the dry fish supplied as diet were ineffective on any stages of *N. rufipes* and no mortality was observed. Durmusoglu *et al*., (2003) had also found that both the formulations (neem oil and neem Azal) did not have enough insecticidal activity on different stages of *Nezara viridula* to control adults and newly laid eggs.

But some authors have opined that plant extracts do show significant ovicidal, insecticidal and larvicidal effect (Raja *et al*., 2003, and JiDong, 2002). Makanjuola (1989) reported an egg mortality of 65% when *D. maculatus* was treated with aqueous neem leaf extract, but in the present study the leaf extracts of *Glyricedia maculata*, *Pongamia pinnata*, *Chromolaena odoratum* seed extracts of *Annona squamosa* and neem seed oil extract (Nimbicidin) were ineffective and did not cause mortality of any stages of *N.rufipes*.

Bruce *et al*. (2004) found that once the larvae of *Sesamia calamistis* and *Eldana saccharina* were feeding inside the stem of maize, the differences between the control and the neem treatments remained similar, indicating that neem ceased to have an effect on survival. Similarly in the present study, because *N.rufipes* was feeding inside the fish, the difference between the control and the plant extract treatment remained similar when the fish fed to the beetle was treated with leaf extracts of *G. maculata*, *P. pinnata*, *C. odoratum*, seed extracts of *A. squamosa* and neem extract and as observed

by the authors in the above study these extracts applied on the surface of the fish may not have any systemic effect.

Studies by many authors have highlighted the antifeedant property of neem extracts (Barnby and Klocke, 1987; Arnason, 1985; Meisner, 1985; Juan and Sans, 2000). Ma *et al*., (2000 a) assumed that repellence of neem leaflets to *Helicoverpa armigera* was an indication of an antifeedant property of neem leaf extracts. Most studies on neem have focused on extracts, such as azadirachtin (Ma *et al*., 2000a), from seed kernels. Several polar and non-polar compounds (limnoids) are believed to be responsible for the antifeedant properties of neem leaves (Jacobson *et al*., 1978). Further studies need to be done to investigate the antifeedant effect of these extracts on the growth and development of *N.rufipes.*

Of the 6 concentrations (10%, 5%, 2%, 1%, 0.5% and 0.25%) of oils tested a concentration dependent mortality was observed. When the 3^{rd}, 4^{th},and adult stages of *N. rufipes* were treated with oils of CLGO, LGO-A, LGO-B, Cinnamon, Clove, Black pepper and Turmeric, the LC50 was highest for 4^{th} instar and lowest for adult beetle. In the case of LGO-A oil, LC50 was highest for 4^{th} instar, and lowest for adult. The LC50 of turmeric oil was highest for 3^{rd} instar and lowest for adults. The higher susceptibility of the adults to the oils when compared to that of the larvae may be related to its physiology.

Don Pedro (1985) found that orange peel was more effective than grapefruit and at an application of 15.0 and 18.0 % by weight; orange peel powder reduced progeny development and slowed larval development. At the maximum applied rate of 18.05%, the number of emerging larvae was reduced by 60%, while in the present study complete inhibition of progeny development of *N. rufipes* was achieved with all the oils and at all test concentrations (10, 5, 2 1, 0.5 and 0.25%).

Observation of the process of hatching revealed that the apparent reduction in successful egg hatch was due to the effect of neem on crawlers after eclosion from viable eggs when they came into contact with the residues on leaves and on the egg chorion. . They observed several of such half emerged dead crawlers. Hence, the reduction was not due to a disruption or inhibition of embryogenesis as reported by Kumar *et al*. (2005). These observations are similar to the one reported by Von Elling *et al* (2002). But the neem based Nimbicidin used in the present study did not inhibit egg hatch.

The oils (CLGO, LGO-A, LGO-B, Cinnamon, Clove, Black pepper and Turmeric oil) used in the present study were extremely effective in killing the 1st and 2nd instars of *N. rufipes,* but Don Pedro (1989) observed that 1st instar development of *D. maculatus* on strips of dried trout fillet (*Salmo gardinerii*) was significantly retarded with oils such as groundnut, traditional coconut, and industrial coconut, palm and shark liver oil only at very high application rate of 112ml oil/ kilogram. The author has also stated that fixed vegetable oils such as groundnut, traditional coconut, and industrial coconut, palm and shark liver oil need to come in to direct contact with the egg to prevent hatching. In this study, all the oils were used as fumigants and there was no need for the oil to come into direct contact with the eggs to prevent hatching.

Okorie *et al*, (1990) obtained 92% larval mortality of *D. maculatus* when dried tilapia was treated with 2% neem seed powder, but in this study, neem extract (nimbicidine) did not cause any mortality, while100% mortality was obtained only when 1st and 2nd instars of *N. rufipes* were treated with 2% concentration of all 7 oils treated, which is an indication that these oils may have better larvicidal activity. It is also possible that *N.rufipes* is more resistant to neem extracts.

Leatemia and Isman (2004) observed growth retardation and

recommended the crude seed extract of *A. squamosa* as a promising candidate as a botanical insecticide, whereas in our study when insects were treated with crude ethnolic seed extracts of *A. squamosa* no mortality was observed.

The concentration of crude aqueous emulsions of ethnolic seed extracts of *A. squamosa* to kill 50% of 3rd and 4th instar larva *P. xylostella* was 5.2% w/v and 8.7% w/v respectively (Leatemia and Isman, 2004), while in the present study 50% of 3rd and 4th instar larval death was obtained at a concentration of 2.30% (turmeric oil) and 1.54% (CLGO) respectively, which is much lower than that obtained in the above study.

The concentration of crude aqueous seed extracts of *A. squamosa* to kill 50% of adult *Orius insidiosus* was 1.95 w/v (Leatemia and Isman, 2004), but in the present study even with the least effective oil (turmeric) 50% of the adult death was obtained at a concentration of 0.35% which is much lower than that obtained by Leatemia and Isman (2004).

In adult *D. maculatus*, LC50 with orange peel was obtained at a concentration of 14.1% after 7 days, but 99% death was obtained with only a higher concentration of orange peel (21.3%v/w) (Don Pedro, 1985), whereas in the present study even with the least effective oil (turmeric), 50% of larval death was obtained at a concentration of 0.349% which is much lower.

Adedire and Lajidae (2000) reported that 10% (w/w) of the *Piper gunieence* on dry fish brought about 100% death of the larvae, pupae and almost 100% death of the adult *D. maculatus* while 10%(w/w) of *Dennettia tripetala* on dried fish killed 100% of the pupae and adult and resulted in 95% death of the larvae of *D. maculatus*, but our results indicate that at a lower concentration (5%) of all the oils tested (CLGO, LGO- A, LGO-B, cinnamon oil, black pepper oil, clove oil and turmeric oil) 100% mortality of all stages of *N. rufipes* was obtained.

When *Callosobruchus maculatus* was treated with 80% of methylated spirit + neem seed extract (Makanjuola, 1989), 57.5% of the eggs hatched and 57% of the adults were dead. In our study, 100% adult *N. rufipes* were dead at 10% and 5% of various oils (CLGO, LGO-A, LGO-B, Cinnamon oil, Clove oil, Black pepper oil, and Turmeric oil) and only 0.002% alcoholic solution of crude lemongrass oil was required to kill 50% of the adults and all oils at all concentration tested (10%, 5%, 2%, 1%, 0.5% and 0.25%) killed 100% of the egg. This again points to the better effectiveness of the spice oils.

In the present study adult beetles of *N. rufipes* were found to be more vulnerable to the toxic effect of various oils compared to the susceptibility of 3^{rd} and 4^{th} instar larvae. From the screening of the five oils and the two fractionation products of Lemon grass oil (LGO-A and LGO-B), crude lemon grass oil was found to be the most effective toxic oil followed by LGO-A, Cinnamon oil, Clove oil, LGO-B, Black Pepper oil and Turmeric oil.

CLGO was observed to cause higher mortality in adults and all larval stages when compared to the other two refined fractions of lemon grass oil, viz. LGO- A and LGO- B, in *N. rufipes.* This shows that the crude oil extract (CLGO) is more effective than the refined oil fractions LGO-A &B). Hence, the above mentioned results indicate that the spice oils tested in the present study are more effective than crude plant extracts recommended by other authors. Simple crude extracts from plants have been used as insecticides in many countries for centuries (Crosby, 1971). Crude plant extracts often consists of complex mixtures of active compounds. Advantages of using complex mixtures as pest control agents are that natural mixtures may act synergistically (Berenbaum, 1985), and they may show greater overall bioactivity compared to the individual constituents (Berenbaum *et al*., 1991; Chen *et al*., 1995). Insect resistance is much less likely to develop with mixtures (Feng and Isman, 1995) and the various bioassay results showed that crude seed extracts of *A. squamosa* have both toxic as well as antifeedant

properties (Leatemia and Isman, 2004). All the above observations support the use of chemically unrefined crude oil containing mixtures of bioactive plant compounds rather than the use of the pure individual compounds. Further more, the crude extracts will be simpler and cheaper to prepare if the plant materials are locally available.

Although in the present study oils have been found to be highly effective in controlling the pest, the mechanism of action is still to be clearly understood. The mode of action of oils is partially attributed to interference in normal respiration, resulting in suffocation (Schoonhoven, 1978). Hewlett (1975) has opined that the most likely mechanism of activity of a refined mineral oil on *Sitophilus granarius* adults was that of blocking the tracheal system, causing beetle death by anoxia. However, factors other than oxygen starvation probably also play a role in their mode of action (Schoonhoven, 1978; Shaaya and Ikan, 1978). Egg mortality has been attributed to toxic components (Su et al., 1972) and also to physical properties, which cause changes in surface tension and oxygen tension within the egg (Singh *et al*., 1978). It is also thought that oils exert some lethal action on developing embryos or first instar larvae, e.g. by the reduction in the rate of gaseous exchange due to a "barrier" effect and /or direct toxicity by penetrated oil fractions (Don Pedro, 1989). Another hypothesis is that oil infiltration under the operculum may block respiration or disrupt the water balance of egg and developing embryos (Messina and Renwick, 1983).

The results of the studies indicate that the alcoholic solutions of various oils such as Crude lemon grass oil, Cinnamon oil, Clove oil, Black pepper oil, and Turmeric oil are effective toxicants, which could be recommended for effective control of the dry fish pest, *N. rufipes*. However, the optimum concentration and exposure time required for large scale applications are to be determined, depending on the specific storage conditions.

CHAPTER VIII

FIELD SURVEY

FIELD SURVEY

The field survey has been carried out with the objective of getting a first hand knowledge about the methods of fish drying and storage practiced in each center, the extent of dependence of fishermen on the trade, the threats faced by this industry, the various pests attacking dried fish and the various indigenous methods of control practiced by the local fishermen.

The information thus gathered will help in devising better control methods and more hygiene ways of fish processing.

Thiruvananthapuram

Large scale as well as small-scale fish drying was observed all along the coastal belt. Dry fish industry is a 5 crore /year turnover business in these regions. Large-scale fish drying begin soon after the monsoon and continue until the onset of monsoon next year. At the first signs of monsoon, fishermen stop large-scale fish drying. Small-scale fish drying continues until monsoon attains its full strength. In addition to the local fishermen, those coming to these coasts from Mangalapuram with sophisticated gears indulge in hectic fish processing activities throughout the season. Large stretches of sandy beaches in the Thiruvananthapuram region help in making the drying process easier. Punthura and adjacent coasts lack sandy beach and here the fish drying was done upon the rocks. Fish drying peaked when there was surplus yield from the sea. Drastic fall in the fish yield has made it difficult for the fishermen community to carry on with their traditional occupation. Sardine was the major commodity available, especially during 'Chakara', for drying. Tamilnadu is a major market for the dried fish where it is used as poultry feed and also for human consumption. Hence, the fishermen in the Thiruvananthapuram coasts get good price for their produce. Madhya

Pradesh, Uttar Pradesh, Gujarat, Maharashtra also form major markets. Excessive use of pesticides like ammonium chloride, BHC, DDT during fish processing as well as during storage to prevent pest attack is observed in the fish drying centres of Thiruvanthapuram. Diarrhea, nausea, and other related ailment are of common occurrence among the fishermen community over here, which may be related to the contamination of the water bodies with these insecticides. Fish for both human consumption and poultry feed were processed here. Fish is dried either directly on the sand or upon the coir mat. Fish for human consumption was gutted and kept on coconut fronds (Plate III, Photo 17) /coir mat for drying. Small scale drying and its marketing were mostly done by women **(**Plate- III, Photo 18). Nets were placed around and on top of the drying area to protect it from being eaten by the birds, cats etc. The storing and drying areas although not hygienically maintained were devoid of any insect pests, which again indicate the possible use of insecticides. Laboratory reared larvae reared on the sand brought from the region died within a day, which further strengthens the information that insecticides are used in these areas for pest control.

Major fish drying centers were : Erayamanthura, Chembakaramanthura, Puthiyathura, Adimala, Pallam, Karimkulam, Kochuthura, Aadimalathura, Cheriathura, Pallithura, Thumba, Saint Antruce, Punthura, Bhimapalli, Valiathura, Thoppu, Shankumukhom, Kannumthura, Cheruvettukad, Cheruvettukad, Valiyavettukad, Cheriaveli, Valiaveli, Puthanthoppu, Vettuthura, Naluthengu, Shantipuram, Marianad, Puthukuruchi, Perumathura, Thalampalli, Puyrhra, Anjuthengu, Poziyuru, Parthur, Pulluvila, Kochuvila, Vizinjam, Kollangode, Eravanthura, Fathimamatha, Ovilorpumatha, Karichankayal to Somatheram (Gramam), Paruthur/ Paruthiyur (TN border), Puvar, Kochupalli,and Charathadi. There were about 150 stocking sheds (chappa) all along the sandy beach from Poovar to karichal kayal.

Major fishes dried were: - Natholi *Stolephores indicus* ; kalaba *Epinepheles tauvina*; prawn; chaala *Sardinella longiceps*; kanava *Sepia pharonis*; powdered prawns, aila *Rastrelliger kanagurta*; kattila *Pristpomoides* spp,; chuvanna kattila *Nemipteres* spp.; chura *Euthjynnus affinis*; koziyala *Selar crumnothalmus*; mural *Epinepheles diacanthus;* and kara *Penaeus monodon*.

Major pests observed were: *Necrobia rufipes, Dermestes maculatus* and *Calliphora* sp. Other insects associated with dried fish were Species H (Elateridae) (Plate IX, Photograph 64), Species F (Carabidae) (Plate VIII, Photograph 62), Species E (Scaraboidea) (Plate VIII, Photograph 61).

Kollam

Neendakara and Shakthikulangara are the main fish catchments areas at Kollam. Large-scale fish drying is not possible here because of the lack of stretches of sandy beaches. Small-scale fish drying of high quality fishes in courtyards attached to the habitations of the fishermen are marketed for human consumption (Plate III, Photo 19). The opened and cleaned fish are laid on coir or jute mat with a net on top to protect them from birds. Sardines marketed as chicken feed, are dried directly on the available sandy stretches. Only in Kollam the sardines were found mixed with sand (Plate III, Photo 21,24), which may delay the drying process . This may be done to prevent insect infestation as the sand was possibly mixed with insecticides as indicated by our laboratory studies. Fish drying centers were quite untidy and unhygienic. About 99% of the fish processed here were for human consumption, which is indeed of great concern in the wake of the observation made on rampant use of insecticides in the field. As there were no chappas here the fish were heaped on the sand itself (Plate III, Photo 20) and sometimes covered with plastic sheets (Plate III, Photo 22**).** Shakthikulangara was marked by the presence of many major shark-processing centers.

Thousands of fishermen directly or indirectly earn their livelihood from the fish drying business. Pathanamthitta and Kottayam are among the major marketing centers. There were about 15 chappas.

Other major fish drying centers : Mariyalayam, Puthanthura, Thankasseri, Vadi, Mathukara, Pallithottam, Port kollam, Chavara.

Major pests observed were: *N. rufipes*, *D. maculatus* and *Calliphora* sp. in very low numbers.

Fishes commonly used for drying were: **-** Mullan *Leognathus* spp. **(**Plate- III, Photo 23); bral *Chana muraleus***;** manthal *Cyanaoglossus semifasciatus***;** Natholi *Stolephores indicus* (Plate IV, Photo 25), Kora *Sciaenids* spp**;** shark *Carchariunus* spp**;** prawn, sardine *S. longiceps*; mackeral *R. kanagurta* (plate IV, photo 26,27)**.**

Alappuzha

Vast stretches of coastal areas were used as fish drying yards. Coir mats are spread on the sandy coast and fishes are dried over it. The sacks were mostly filled in the open (Plate V, Photo 37). There were about thirty Koodam (Chappa) (Plate V, Photo 35, 38) along the coasts from Thottappalli to Valizazeekkal, Thaikkal, Mararikkulam, and Omanappuza coasts, which are used as the temporary storing place for dried fish. High demand for the commodity left no room for stocking the commodity for long, hence the few number of chappas. Thousands of labourers depend on the dry fish industry for their livelihood. Low fish catch was the major problem that threatens the dry fish industry in Alappuzha**.** DDT and other insecticides were found to be used extensively to prevent insect pest infestation. In addition to mixing with the sand upon which fishes were spread for drying, insecticides were also dusted on sacks in which dried fishes are packed (Plate V, Photo 36). All along the coastal belt from Thottappalli to valizazeekal (about 25 km), fish

drying practices are in full swing throughout the season beginning from August to May. In Alappuza coastal belt, the Chakara is not a constant phenomenon pertaining to a particular region and it can shift, which compels fishermen to move from one area to another in search of a bulk yield. For these reasons, permanent fish drying centers were absent in these regions. Fish drying for human consumption depends on availability of commercially valuable fish for a moderate price. Steady hike in the price has left fishermen with no option other than drying cheaply available fish for poultry feed. The difference noticed here when compared to other fish processing centres in Kerala is the cleanliness in processing practices. Fishes were spread over the coir mat and coconut fronds (Plate IV, Photo 30,33 and 34), which prevent mixing of the dried fish with the mud, sand etc. The dried fish were sometimes stored in the beach itself and coir mats were used to cover them. (Plate IV, Photo 28). Clean dried fish fetch more money for the fishermen. 'Kudumbasri' (NGO) provide labourers for the chappa owner. Three wards, namely; Therayilkadavu, Perumpalli, and valizazeekkal known for intensive fish drying activities were devastated by the tsunami which hit Alappuza and Kollam districts in 2001. Sandy beaches disappeared; fish yield declined which adversely affected the dry fish industry. Years after the tragedy, the situation has improved and slowly and steadily the fish drying activities were gaining momentum.

Major fish drying centers were: North: - Purakkad, Punnapra, Kurishumpallikkal, Esa, Nadakkal, Vattayal, Kanchiramchira, Omanappuza, Kattur, Mararikkulam, Chethi, Cheriavali, Arathingal, Thaikkal, Manakodum, Puthithodi, South:-Varakkad, Valanjavazi, Ambalappuza, Thottappalli, Pallana, Thrikkunnappuza, Arattuvazi,, Kallikkad, Kayamkulam, Vadakkal, Kakkazam, Vattachal, Perumpalli, Therayilkadavu, Mangalam, Arattupza (Pathisseri), Cherthala, Anthakaranazi.

Major pests collected: *N. rufipes*, *Calliphora* sp., and *D. maculatus* were collected from the storing sheds along with Species I (Tenebrionidae) (Plate IX, Photograph 65).

Major fish available for drying process were: sardine *S. longiceps*; chuda *Escolosa thoracata***;** mackeral *R. kanagurta*; thala *Trichurus lepturus***;** natholi *Stolephores indicus* and manthal *C. semifasciatus***.** Prawns were also dried in the cost in large quantities (Photo IV, Photo 29).

Ernakulam

Vypin

About fifteen fish drying yards and stocking sheds were located adjacent to each other. Hundreds of such yards were there until a few years back. A steady decline in the fish yield and consequent hike in the price left this business unaffordable and many quit the field**.** The fishermen in this area were specialized in mackeral processing. They were processed in two different ways. In one method, fishes with their operculum, gills and intestinal parts removed and cleaned were spread in layers with salt alternating in concrete tanks (Plate V, Photo 39,40). An average of 2 kg salt / 1 kg of fish were used. After 2-3 days the fishes were removed from the tank and cleaned well, spread on coir mats over the floor of the yard (Plate VI, Photo 41). Processed fishes were packed in coconut leaf mat and are transported to various parts. About twenty workers were engaged in a single fish processing shed and attached yard. Many more are indirectly engaged in the fish processing and allied work. Another processing technique practiced here is to keep the fish in tanks filled with saturated salt water, for about 1 week and then taken out and the salt-water drained off (Plate VI, Photo 42). After proper drying fishes were cleaned and packed in bamboo containers (Kotta), which enclose about 44 kg of fish, to which 8 kg salt were added. The major

markets for the processed fish are Parinthalmanna, Trichur, Palghat, Aluva, kottayam, Athirampuza, Changanassery, Ernakulam, Kodungallur etc. in Kerala and Trichi (especially Velipuram), Chennai etc in Tamil Nadu. Chemban (keziyali), vaala, ribbonfish etc are considered as quality dried fishes. The processing sheds were concrete structures with well-plastered walls and floors without any crevices. The processing method practiced here resulted in very low incidence of beetle pest. Major season for the fish processing were August to June and the peak season January to April. In addition to Vypin fish processing was also carried out in Munambam, and Kunhithi.

Insects collected from the storing sheds: *Necrobia rufipes*, and *Calliphora* sp., Species H (Elateridae) (Plate IX, Photograph 64).

Fishes processed for human consumption: Vaala *Chirocentrus* spp**,** Manthal *C. semifasciatus*; Kora *Sciaenids* spp.; Pallikkora *Ottolithus* spp.; Kadal bral *Rachycenton canadus***;** Mackeral *R. kanagurta,* Olanchi Manungu *Engraulis* spp.**;** Nandan *Ambasis***;** Etta *Arius* spp.**,** etc

Azeekkode

Azeekkode beach was well known for fish drying practices until recently. As the government has declared the beach as a tourist spot and banned fish processing on the beach, fishermen engaged in fish drying related job are facing unemployment and there were widespread discontent over the new policy of the government. The government order came as an unexpected blow to the already ailing dry fish industry in the region. Until 2006 there were about ten sheds. Sardine and other relatively cheap fishes were extensively dried and exported from here to different parts of the country.

Trichur

Chavakkad

Entire beach stretches for about 10 kms and extend up to the Ponnani coast. Sardine processing was carried out on a large scale.. There were about 60 chappas along the coasts of Puthankadappuram, Edakkayur, Manthalamkunnu, Adathode, Palappetti. Indiscriminate use of insecticides during fish processing may be the reason for the relatively low pest attack. Laboratory studies have also pointed to this possibility.Major exporting centers are Tamilnadu, Andhra Pradesh, and Karnataka etc. Drying fishes for human consumption stopped due to unavailability of the high quality fishes and consequent hike in the price. Infection reaches its peak during monsoon season due to improper drying**.** Approximately 600 families were directly or indirectly connected with the dry fish industry.

Major fish drying centers were: Puthankadappuram, Edakkayur, Manthalamkunnu, Adathode, Palappetti, veliyamkode (which merge with Ponnani coast), and Nattika.

The common insects observed along with the dried fish were: *N. rufipes*, *Calliphora* sp.,and *Dermestes* spp., Species A (Histeridae) (Plate VIII, Photograph 57) and Species K (Ephydridae) (Plate IX, Photograph 67).

Fishes processed: Mackeral *R. kanagurta*, thala *Trichurus lepturus*; natholi *S. indicus*; mullan *Leognathus* spp.; manthal *C. semifasciatus*; malan *Mugil* spp.; veluri *Escolosa thoracata*,

Malappuram

Tirur, Tanur and Parappanangadi

Vast stretches of beaches help in easy fish drying. About forty chappas were located all along these beaches. The chappas were temporary structures

made of bamboo poles and thatched roof. The sides were covered with pleated palm fronds. Fish was dried directly on the sandy beach. The beetle pests (both larvae and adults) in large numbers were found in the moist sand beneath the coir mat inside the chappa. The pests moved easily from here in to the sacks packed with dried fish when it was stored here. During peak season, about three trucks/week leave from each coast to different destinations. No obvious application of insecticides were noticed (laboratory tests also confirmed this.) which render the dried fish prone to intense pest attack. An interesting observation was the presence of *N. ruficollis*, which is the first report of this dry fish pest from India. Histerid beetles have been reported as predators of *D. maculatus* (Haines and Rees, 1989). Unlike in the southern districts where small scale drying is common, fish drying here is monopolized by a few people employing large number of labourers. About two thousand labourers were engaged here in the fish drying and related works. Sardine was the most common fish available for drying. Fishes for human consumption were rarely processed here due to non-availability and unaffordable price of the highly priced varieties. Fish oil extracting centers were found to operate on these coasts and oil extraction is being done on a limited scale. A couple of years back, large-scale extraction was being done, but because of the problem of waste disposal and consequent opposition from local population it has been scaled down. Mostly sardines are used in oil extraction (Plate VI, Photo 48; Plate VII, Photo 49). A large number of *N. rufipes* possibly feeding on the dried oil were found on the wooden planks- used to cover the concrete tanks in which oil extraction was done- piled outside the extraction shed.

During 2006 when bird flu struck the chicken farms of Tamilnadu, dried fish stocks piled up here (as in majority of the large scale fish drying centers), due to the low demand for chicken incurring huge loss. Situation has

improved and the fish processing has continued unabated since then. Fish oil extraction is being done here although on a limited scale.

The chappas piled with sacks of dried fish were found to be heavily infested with *N.rufipes Calliphora* sp. and *Dermestes maculatus*.

Other beetle species observed in association with dried fish were: Species A (Histeridae) (Plate VIII, Photograph 57), Species B (Histeridae) (Plate VIII, Photograph 58), Species C (Histeridae) (Plate VIII, Photograph 59), Species K (Ephydridae) (Plate VIII, Photograph 67), Species H (Elateridae) (Plate IX, Photograph 64), Species F (Carabidae) (Plate VIII, Photograph 62), Species I and J (Tenebrionidae) (Plate IX, Photograph 65,66), Species G (Anobiidae) (Plate IX, Photograph 63), Species E (Scaraboidea) (Plate VIII, Photograph 61) and Species D (Dytiscidae) (Plate VIII, Photograph 60).

Fishes dried: Sardines *S. longiceps;* mackeral *R. kanagurta;* natholi *Stolephores indicus***,** manthal *C. semifasciatus***.**

Ponnani

Ponnani is a major fish-drying center in Malappuram district. Jangar and Marappalam are the two major fish drying centers. Fish reach at Jangar port and the major part is dried on the vast stretches of beach available there. A portion is taken to Marappalam where they are dried and stocked in sheds. Dried fish from the Jangar region are also stocked in the sheds at Marappalam.There are about 45 sheds at Marappalam which are different from those found at Puthiyappa, Tanur, Tirur and Parappanangadi. All the sheds were concrete structures with tiled roof (Plate VI,Photo 46). The floor of the shed was partially concreted. Fish salting was also carried out inside the shed, which encloses a number of tanks for the fish processing in saturated saline water. The fish drying yards were located in between the stocking

sheds at Marappalam where as vast stretches of fish drying yards along with thatched stocking sheds (Plate VI, Photo 44, 45) close to the sea shore were located at Jangar port. The sheds were very long structures. The premises of the drying yards as well as the stocking sheds were found to be heaped with the fish refuse which contributed to the insect infestation in the area. Accumulated fish waste near the stocking shed form a perfect breeding ground for insect pests when there is no fish stock in the stocking shed (Plate VI, Photo 43,47). Unscrupulous application of BHC has been observed in this area. Processors mix BHC with the sand upon which the fish is dried. The floor of the shed and dried fish filled sacks were also found to be dusted with BHC. Our laboratory tests have confirmed the presence of insecticides in the sand collected from this location.

Fishermen of about 500 families were directly or indirectly involved in the fish drying and related works. The sharp decline in the fish catch over the years has negatively impacted the dry fish industry. The fishermen community faces unemployment. High percentage of illiteracy and inability to do any other skilled job has prevented the younger generation from getting employed elsewhere. Chellu fetch only half the price of the sardine in the market. About 8-load/ week were exported to the neighbouring states such as Tamil Nadu, Andhra Pradesh, and Karnataka.

The major fish dried was: sardine *S. longiceps and* 'Chellu' which is a mixture of small sardine *S. longiceps*, squilla, crab, and small prawns was another major commodity for processing **.**

The major pests observed here were: *Necrobia rufipes*, *Calliphora* sp., and *Dermestes maculatus.* Other insects collected from the dried fish stocks were Species H (Elateridae) (Plate IX, Photograph 64), Species F (Carabidae) (Plate VIII, Photograph 62), Species I and J (Tenebrionidae)

(Plate IX, Photograph 65,66), Species G (Anobiidae) (Plate IX, Photograph 63), Species E (Scaraboidea) (Plate VIII, Photograph 61).

Kozhikode

Puthiyappa

The presence of a fishing harbour, makes sure of the availability of the fish for drying throughout the season (August to May) and make it one of the major fish drying centers in Kerala. There are about fifty Chappas here, which are very badly maintained. There are no drainage facilities and wastewater gets logged making the surroundings of the chappa very unhygienic. Most of the chappas are walled structures with cement flooring and thatched roofing. But the crevices on the floors and walls (Plate VII, Photo 52, 53) due to damage to the plastering were used as resting places by the insects when fish was not stocked in the yard. Hence, a residual population of the pests is always present in these yards and they start a new cycle of infestation when fish is stocked in these sheds. Fishes were dried in the open sandy shore as well as on specially prepared yards with cemented floor (Plate VII, Photo 51) which does prevent pest attack during drying, but this advantage is offset due to the badly maintained stocking sheds which provide conducive conditions for pest attack (Plate VII, Photo 50). Properly dried fishes were filled in the sacks (Plate VII, Photo 54) and exported to adjacent states depending on the demand or else stored in the chappa. Sardine is the most common fish dried here which are primarily meant for poultry feed. Fishes for human consumption were also dried and exported to national and international markets. Salting in tanks followed by cleaning and drying was another technique observed here. Some fishermen here have taken up marketing of dried fish for human consumption in a big way. Attractive and hygienic vacuum packaging of the products practiced by some people will help in increasing the market share of dried fish. About 4000 labourers are directly or

indirectly employed in the fish drying and associated work. Use of insecticides is almost negligible as shown in our laboratory studies, where larval mortality was also not significant.

Common insect pests observed in Puthiyapa: *N. rufipes*, *Calliphora* sp., and *D. maculatus* Other associated insect species were Species A (Histeridae) (PlateVIII, Photograph 57), Species B (Histeridae) (PlateVIII, Photograph 58), Species K (Ephydridae) (Plate IX, Photograph 67), Species H (Elateridae) (Plate IX, Photograph 64), Species I (Tenebrionidae) (Plate IX, Photograph 65), Species G (Anobiidae) (Plate IX, Photograph 63) and Species E (Scaraboidea) (Plate VIII, Photograph 61).

Fishes dried: Sardine *S. longiceps*; Mackerel *R. kanagurta*; nathal *Stolephores indicus***;** shark *Carchariunus* spp.; thala *Trichurus lepturus*; puffer fish *Tetrodon* spp.; mullan *Leognathus* spp**.**; manthil *C. semifasciatus*; adavu *L. delicatulus* etc.

Kasaragod

The fish-drying season here begins in August and last until the arrival of monsoon. There were about sixty fish stocking sheds. About 5000 individuals are directly or indirectly engaged in fish processing. In this area, about 90% of small scale drying is done by women (Plate VII, Photo 56). Fish oil extraction was done in a big way and there were well-equipped fish oil mills for doing full-scale oil extraction (Plate VII, Photo 55). The fish drying was done on the vast sandy beaches. Fishes were spread on coir/jute mat for drying and were well protected by the nylon net. On an average 15 truckloads of fish are transported from here in a week. Relatively low-level pest infestation in the field could be attributed to the rampant use of the DDT and other insecticides. One sack was filled with 30 kg of dry fish. One load consisted of 300 to 315 sacks.

Common pests observed were: *N. rufipes*, *Calliphora* sp. and *D. maculatus*. Other insects collected from the drying centres were Species K (Ephydridae) (Plate IX, Photograph 67), Species H (Elateridae) (Plate IX, Photograph 64), Species J (Tenebrionidae) (Plate IX, Photograph 66).

The major fishes processed were sardine *S. longiceps*; mackerel *R. kanagurta*; Natholi *Stolephores indicus***;** mullen *Leognathus* spp.; manthal *C. semifasciatus***;** adavu *L. delicatulus***;** malan *Mugil* spp.; etta *Arius* spp.; veluri *Escolosa thoracata* and kora *Sciaenids* spp**.**

Summary

Traditional fish processing is an important livelihood activity for large numbers of poor people in many tropical developing countries. Traditionally processed fish includes fish that are salt dried, wet salted, dried without salting and smoked. Despite a general decline in traditional fish processing over recent years, there remains a strong market demand for traditional fish products which continue to ensure livelihood and food security for a substantial number of poor dried fish producers and consumers.

For poor in fishing community, fish processing offers an opportunity for income generation that requires relatively low investment. Being a traditional household-level activity, it is relatively easy for poor to acquire the skill and social approval necessary to take up fish processing.

Large scale as well as small scale traditional fish drying was observed all along the coastal belt in Kerala. A large majority of people involved in traditional small-scale fish drying are women. Small scale fish drying is common in the southern districts of Kerala, viz. Thiruvanthapuram, Kollam, Alapuzha, Ernakulam and Trichur. Whereas, the large-scale fish drying was carried out by men and women together. In almost all fish drying centers across Kerala, large stretches of sandy shores facilitate fish drying. Chappa

(thatched huts as well as concrete structures) form the temporary stocking place. Hundreds and thousands of fishermen along with a large section of the society work hand in hand and find their livelihood from the fish dying industry.

Traditional fish drying is adversely affected by insect infestation. *N. rufipes* is one among the major pests of dry fish and a major cause of harvest loss of traditionally cured fish in Kerala. For small-scale processors it is a particularly serious problem that threatens livelihood security, and often has a far-reaching consequence upon socio-economic well being. Various insect pests were found to attack the fish at different stages of processing. Consequently the pest population of a particular processing center was found to vary. Other undesirable factors, which adversely affect the traditional fish drying industry, include monsoon rains, inadequate processing and storage facilities and limited access to transport.

Use of the coir mat for fish drying in the Alappuza coastal area and concrete flooring for fish drying in the Puthiyappa reduce chances of insect infestation to some extent.

Unscrupulous use of insecticides was observed in almost all coastal areas except Puthiappa, Tanur, Parappanangadi and Tirur, which pause serious concern for human health. Untidy and unhygienic conditions in the fish curing centers not only promote insect infestation but also raise serious health problems among the fishermen community.

Highly priced fishes such as Naimeen *Rachcentron canadus***,** shark *Carchariunus* spp**,** mackerel *R. kanagurta***,** Natholi *Stolephores indicus***,** adavu *L. delicatulus***,** thala *Trichurus lepturus* etc were dried for human consumption where as relatively cheap and surplus fishes such as sardine etc were dried and sold as poultry feed. Major export destinations are the adjacent states

such as Tamil Nadu, Andhra Pradesh, Karnataka and other states like Maharashtra, Gujarat, and Madhya Pradesh.

A periodic decline in the fish catch threatens the traditional fish drying industry in Kerala. Widespread pollution of the water body and the trawling has resulted in the depletion of the marine fish resources.

Factors which can influence *N.rufipes infestation* levels

Factors associated with the processing methods used and external conditions at the processing site increase the chances of *N.rufipes* attacking the fish. This may be because the conditions favour the survival of the eggs or larvae, or because the fish is more attractive to the female *N.rufipes.*

Factors associated with the processing methods

Preparation

Removing the scales of fish before drying will deny oviposition sites for the beetles (in the present study it was observed that *N.rufipes* laid eggs mostly under the scales where enough is available). Quality of the raw fish is important. Only fresh fish should be processed. Processing partially spoiled fish will increase the likelihood that *N.rufipes* will attack the fish after sun drying, as it provides more crevices for egg laying.

Drying and stocking

Infestation was found to be prevalent more among the fishes, which are dried upon the ground and stored in temporary sheds (for eg; Puthiyappa, Tirur, Tanur and Parappanagadi etc). Infestation could have been less if the fish were dried upon the raised racks. Drying the fish upon raised racks also improves the rate of drying, as the air can circulate around the fish and can reduce indirect infestation. Drying fish directly upon sand or loose earth as is

done at many drying centres across Kerala is an even greater problem because the larvae are able to burrow into the sand or earth when temperatures are high and move back into the fish again once the temperature begins to fall. Drying fish on sand or loose earth therefore actually helps the larvae to continue infesting the fish.

Where it is not possible to dry the fish off the ground, clean mats or sheeting should always be placed beneath the fish to protect it from indirect infestation. Drying the fish on cement platforms is another method to prevent easy access and deny hiding places for *N. rufipes* and other pests. The advantage of storing dried fish in concrete sheds with cement flooring and well plastered walls and roof is that it does not provide crevices for the beetles to hide when the shed is cleaned. Such hiding places will help the pest to reinfest the fresh fish stocks. In temporary structures, the sandy floor, the pleated coconut fronds- which forms the wall and the roof of the shed – and the coir matting which covers the sandy floor, provides a temporary refuge for the different stages of *N. rufipes* and it is easy for the pest to infest fresh stock of dried fish when the sheds are stocked again.

The preset studies have also indicated that providing illumination in the stocking sheds when the fish is stocked can reduce the fecundity, longevity and food consumption of the pest thus reducing the infestation.

External factors: -

Season: During monsoon there is a marked decline in the *N.rufipes* infestation. This may be due to the low temperature in the stocking sheds. (The present study has indicated that development is slower, mortality high and fecundity low at 25^0C).

Hygeine standards at processing sites

Allowing fish waste and other refuse to accumulate at processing sites increases the chances of the fish becoming infested with *N.rufipes*. Apart from attracting adult *N.rufipes* into the area, this provides excellent breeding material, thereby supporting population replacement at the site, and increasing the risk of larval cross- infestation onto the processed fish. Processing sites should be kept free of fish waste and other wastes at all times. Fish waste should be collected and disposed off away from the processing area. Cement flooring and plastered walls at fish processing centers both for drying and storing were found to contribute in curtailing the infestation as it does not provide crevices, which can serve as refuges for *N.rufipes* when the shed is cleaned before restocking.

In areas where fish oil extraction is carried out the wooden planks used to cover the tanks should be cleaned of all oil flakes and dried in the sun before being used again. *N. rufipes* adults were found in large numbers on flake encrusted planks stocked in the shed.

Socio- economic conditions

The failure of processors to adopt remedial measures other than insecticidal control may be due to lack of technical knowledge or could be a considered response to the socio- economic environment within which they operate. If control measures are to be successfully introduced it is essential that any socio-economic constraints to their implementation are fully understood and taken into account.

The socio- economic conditions, which help in or hinder the introduction of improved technology, are:

The costs and revenues associated with improved techniques: - The revenues are associated with the quantity and price of the product sold. If there is any *N.rufipes* infestation there will be a loss of quantity during processing, so reducing infestation is certain to result in a gain in the quantity of the product. The average price per unit weight at which the fish is sold is also likely to be higher since there will be less low quality and more high quality product in the total output. This depends on the premium that consumers place on good quality fish. The purchasing capacity and preferences of the target groups of consumers determine the price of the product. Consumers of dried fish in Kerala are usually the middle class and the poor. This means that their ability to pay a higher price for a better quality product is limited. It may be that in some local markets the price premium is not sufficient to justify the extra investment in time and money.

Interventions to improve fish drying are not cost free and the processor will balance the extra revenue against the costs in determining whether to undertake the investment. Small scale traditional fish processing is essentially an activity undertaken by poor people who are short of financial capital and whose ability to invest on improving their system is very limited. In order to maximize their returns, they are often forced to reduce expenditure and make compromises on quality.

It is probable that social pressures will exercise some influence on the working of the processors. For new techniques to be adopted, the processors have to be open to new ideas and willing to experiment.

Generally, fish processing is carried out in common property lands or open access lands, and very few processors own the processing areas where they work. So disputes may arise about construction of semi permanent structures on common land. Conditions that permit proliferation of *N.rufipes* infestation are the result of a whole range of factors that are often beyond the

control of individual processors. Hence all processors in an area should follow a uniform pattern of processing methods based on techniques to prevent pest attack. This suggests a need for more concerted actions, involving both the immediate stakeholders and relevant support agencies, to ensure that their problem is addressed.

Increased competition for fish and reduced catches also influence the ability of processors to maintain their traditional practices. With traditional fish processing itself increasingly coming under threat as fish catches themselves are threatened, the financial risk faced by the processors run are also increasing. All of these factors, costs and revenues, financial capital, social and natural capital, will influence the motivation of the processors to reduce *N.rufipes* infestation and need to be taken into account when designing control strategies if sustainable livelihoods are to be improved.

In conclusion we can say that while the cost of interventions is not very high, the reduction in losses will be substantial. Improved quality of food will make sure that the fish sells faster in the market.

CHAPTER IX

SUMMARY

SUMMARY

The information available about the dry fish industry, pest infestation of traditionally cured fish by flies and hide beetles and the possible control measures other than the chemical methods is very meager and is evident from the available literature.

This is the first report on the biology of *N. rufipes* - a major pest of dried fish - from India. *N. rufipes* complete their development cycle in 6 instars. It is observed that development is fast and mortality is lower at 32.5^0C, L: D 12:12, than at any other temperature-photoperiod combination. Development at all photoperiods at 25^0C prolongs development and increases mortality, which indicates the unsuitability of this temperature for normal development of *N. rufipes*.

N. rufipes female lived longer than the male and maximum longevity was attained at 32.5°C than at any other temperatures (viz; 25, 30, and 35°C) tested. Though the increase in temperature up to 32.5°C increased the longevity, it declined at 35°C.

The reason for the high infestation of *N. rufipes* during the summer, when temperature in the stocking shed is in the range 30-33°C, can be explained by the results of the present study, where the ideal temperature for growth of *N. rufipes* was observed to be 32.5°C. A steep decline in the population of the beetle during rainy season when the temperature hardly reaches 25°C inside the stocking shed could also be explained on the basis of the results of the present study.

This is the first study of its kind to determine the fecundity of this pest on a diet of dried fish and also to understand the effect of temperature and photoperiod on fecundity.

The results indicate that 32.5°C is the preferred temperature at a photoperiod of L: D 12:12 to realize higher fecundity when compared to the temperatures 25°C and 30°C. It is also observed that exposing the insects to a photoperiod of L: D 24:00 at 25°C and 30°C does decrease fecundity. The longest oviposition period was observed at 32.5^0C, L: D 12:12 and the shortest at 25^0 C; 24:00.

Spreading the dried fish on the floor and packing it only when it is ready for transportation may help in preventing the proliferation of the pest, as the ideal conditions are not provided. Although it may not be economically feasible to store dried fish at below 25^0C, the results suggest that complete lighting of the storage sheds could lead to decrease in fecundity of the pest and hence contribute to its control.

It was also observed in *N. rufipes* that within a temperature regime (either 25°C or 30°C) a photoperiod of 00:24 were the ideal one to realize the maximum fecundity. This again indicates that *N. rufipes* is adapted to darkness and will thrive in such conditions.

The present study reveals that the highest food consumption (0.371111g) was observed at a temperature–photoperiod combination of 30°C and L: D 00:24. The photoperiod L: D 00:24 was the ideal photoperiod for better food consumption among all the photoperiods tested at 30 °C. At 30 °C and L: D 12: 12, 24:00 and 00:24, there was an increase in food consumption from the 1^{st} instar to the 4^{th} instar.

The total amount of food consumed by all the instars at 32.5 °C (0.367342g) was observed to be greater than at all other temperatures tested viz; 35 °C and 30°C at a photoperiod of L: D 12:12. But, at 35 °C only 0.170257g of dried fish was consumed which was the lowest. This indicates that at L: D 12:12, the photperiod that is commonly met within coastal areas

of Kerala, 32.5 °C is the ideal temperature for maximum food consumption. But this is lower than the total food consumption (0.37111g) at 30°C, 00:24.

In this study leaf extracts of *Glyricedia maculata*, *Pongamia pinnata*, *Chromolaena odoratum*, seed extracts of *Annona squamosa* and Neem extract (Nimbicidin) topically applied on the dry fish supplied as diet were ineffective on any stages of *N. rufipes* and no mortality was observed. Alcoholic solutions of various oils such as Crude lemon grass oil, Cinnamon oil, Clove oil, Black pepper oil, and Turmeric oil are effective toxicants, which could be recommended for effective control of the dry fish pest, *N. rufipes*.

Drying fish directly upon sand or loose earth as is done at many drying centers across Kerala is one of the major concerns because the larvae are able to burrow into the sand or earth when temperatures are high and move back into the fish again once the temperature begins to fall.

Where it is not possible to dry the fish off the ground, clean mats or sheeting should always be placed beneath the fish to protect it from indirect infestation. Drying the fish on cement platforms is another method to prevent easy access to *N. rufipes* and other pests

The advantage of storing dried fish in concrete sheds with cement flooring and well plastered walls and roof is that it does not provide crevices for the beetles to hide when the shed is cleaned. Such hiding places will help the pest to infest the fresh fish stocks. In temporary structures, the sandy floor, the pleated coconut fronds- which forms the wall and the roof of the shed – and the coir matting which covers the sandy floor, provides a temporary refuge for the different stages of *N. rufipes* and it is easy for the pest to infest fresh stock of dried fish when the sheds are stocked again.

Fish waste should be collected and disposed off away from the processing area. Cement flooring and plastered walls at fish processing centers both for drying and storing were found to contribute in curtailing the infestation as it does not provide crevices which can serve as refuges for *N.rufipes* when the shed is cleaned before restocking.

CHAPTER X

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PLATE I

Photograph 1- Egg of *Necrobia rufipes*

2- 1st instar of *N. rufipes*

3- 2nd instar of *N. rufipes*

4- 3rd instar of *N. rufipes*

5- 4th instar of *N. rufipes*

6- Anterior portion of 4th instar of *N. rufipes* with head capsule

7- Posterior portion of 4th instar of *N. rufipes* with abdominal cerci

8- Early pupal stage of *N. rufipes*

PLATE II

Photograph 9- Late pre-pupal stage of *N. rufipes*

10- Pupal stage of *N. rufipes*

11- Exposed pupa of *N. rufipes* (Ventral view) with shriveled caste skin of the larva

12- Exposed pupa of *N. rufipes* (Dorsal view) with shriveled caste skin of the larva

13- Head portion of *N. rufipes* with partially sclerotized mandibles

14- Teneral adult of *N. rufipes*

15- Adult male of *N. rufipes*

16- Adult female of *N. rufipes*

PLATE III

Photograph 17- Mackeral being dried on coconut palm fronds at Puthiyathura (Thiruvananthapuram)

18- A small scale fish farmer selling her fish in the market (Vizinjam, Thiruvananthapuram)

19- Small-scale fish drying in front of the household of fishermen (Kollam)

20- Dried fish heaped on the sand in the curing yard at Neendakara (Kollam)

21- 24 – Sardines mixed with sand being dried at Neendakara (Kollam)

22- Dried fish stored in the curing yard covered with plastic sheet

23- *Leognathus* spp. spread on coir mats for drying at Neendakara, Kollam

PLATE IV

Photograph 25- *Stolephores indicus* being dried at Neendakara, Kollam

26 & 27 – *Rastrelliger kanagurta* being dried at Puthanthra, Kollam

28- Dried fish stored on the beach and covered with coir mats at Purakkad, Alapuzha

29- Small prawns being dried at Punnapra, Alapuzha

30 & 33 & 34- Use of coir mats and coconut fronds for drying prevalent in Purakkad, Alapuzha

31- Lorry loaded with dried fish at Purakkad, Alapuzha

32- Sardines being dried on large scale at Kurishumpallikkal, Alapuzha

PLATE V

Photograph 35 & 38- Dried fish storing sheds (Chappas) at Thottapalli, Alapuzha

36- BHC powder dusted on dried fish filled sacks stored in sheds at Thaikkal, Alapuzha

37- Sacks being filled with dried fish at Thaikkal, Alapuzha

39-40- Fish treated with salt in tanks at Vypin, Ernakulam

PLATE VI

Photograph 41- Salted fish from tanks being dried on concrete floor at Vypin,

42- Fish treated with saturated salt water in tanks at Vypin, Ernakulam

43 & 47- Fish waste dumped in the vicinity of the stocking shed at Ponnani, Malappuram

44 & 45 – Thatched stocking sheds at Jangar port, Ponnani

46 – Concrete dried fish stocking sheds Marappalam, Ponnani

48- Oil extraction from sardines at Tirur, Malappuram

PLATE VII

Photograph 49- Oil extraction from sardines at Tanur, Malappuram

50 – Badly maintained stocking shed at Puthiyapa, Kozhikode

51- Cemented drying yards at Puthiyapa, Kozhikode

52 & 53- Crevices on the walls, which provide refuges for the pest at Puthiyapa, Kozhikode

54- Dried fish stored in sacks at Puthiyapa, Kozhikode

55- Full-fledged oil extraction units at Kasargode

56- Women involved in small-scale fish drying in for human consumption Kasargode

PLATE VIII

Photograph 57- Species A (Coleoptera:Histeridae)

58- Species B (Coleoptera:Histeridae)

59- Species C (Coleoptera:Histeridae)

60- Species D (Coleoptera:Dytiscidae)

61- Species E (Coleoptera: Scaraboidea)

62 – Species F (Coleoptera:Carabidae)

PLATE IX

Photograph 63- Species G (Coleoptera:Anobiidae)

64- Species H ((Coleoptera:Elateridae)

65- Species I (Coleoptera:Tenebrionidae)

66- Species J (Coleoptera:Tenebrionidae)

67- Species K (Diptera:Ephydridae)

Table 1- Percentage hatch of eggs of *N.rufipes* at various temperatures and photoperiods

Temperature(^{0}C)	Photoperiod L:D	Percentage of hatching on different days					Total
		unhatched	2 days	3 days	4 days	5 days	
25	12:12	10.00	55.00	31.67	1.67	1.67	100.00
	24:0	13.33	35.00	31.67	6.67	13.33	100.00
	0:24	6.67	66.67	23.33	3.33	0.00	100.00
30	12:12	5.00	78.33	16.67	0.00	0.00	100.00
	24:0	8.33	63.33	28.33	0.00	0.00	100.00
	0:24	3.33	73.33	23.33	0.00	0.00	100.00
32.5	12:12	0.00	86.67	13.33	0.00	0.00	100.00
35	12:12	10.00	90.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 65.8930, df=22, p=.000003

Table 2 - Percentage of individuals of *N.rufipes* that completed first instar at different temperatures and photoperiod

Tempera-ture(^{0}C)	Photo-period L:D	Percentage of individuals that completed I Instar on different days																					Total
		Dead 0	2	3	4	5	6	7	8	9	11	12	14	15	16	17	19	21	23	24	27	29	30	
25	12:12	42.59	0.00	3.70	1.85	9.26	12.96	12.96	1.85	3.70	1.85	0.00	0.00	1.85	0.00	1.85	0.00	0.00	1.85	0.00	1.85	0.00	1.85	100.00
	24:0	53.85	0.00	0.00	1.92	3.85	1.92	5.77	9.62	7.69	0.00	5.77	3.85	0.00	0.00	1.92	0.00	0.00	0.00	1.92	0.00	1.92	0.00	100.00
	0:24	25.00	0.00	8.93	0.00	16.07	23.21	3.57	0.00	8.93	1.79	0.00	3.57	1.79	3.57	0.00	1.79	1.79	0.00	0.00	0.00	0.00	0.00	100.00
30	12:12	7.02	0.00	0.00	0.00	22.81	70.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	14.55	0.00	0.00	0.00	14.55	56.36	14.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	0:24	8.62	0.00	0.00	15.52	75.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
32.5	12:12	1.67	0.00	86.67	11.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
35	12:12	9.26	9.26	81.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 394.062, df=102, p=0.00000

Table 3 -Percentage of individuals of *N.rufipes* that completed second instar at different temperatures and photoperiod

Temperature(^{0}C)	Photoperiod L:D	Percentage of individuals that completed II Instar on different days														Total
		Dead 0	4	5	6	7	8	9	10	11	12	13	14	15	17	
25	12:12	35.48	0.00	0.00	6.45	3.23	3.23	0.00	16.13	25.81	6.45	0.00	3.23	0.00	0.00	100.00
	24:0	16.67	0.00	0.00	0.00	8.33	4.17	4.17	16.67	0.00	0.00	16.67	0.00	25.00	8.33	100.00
	0:24	11.90	0.00	0.00	21.43	4.76	0.00	19.05	9.52	30.95	0.00	2.38	0.00	0.00	0.00	100.00
30	12:12	7.55	1.89	9.43	35.85	26.42	18.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	10.64	0.00	6.38	23.40	34.04	25.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	0:24	0.00	5.77	7.69	32.69	30.77	23.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
32.5	12:12	5.08	10.17	11.86	37.29	33.90	1.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
35	12:12	0.00	4.08	4.08	20.41	34.69	36.73	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 317.578, df=67, p=0.00000

Table 4 -Percentage of individuals of *N.rufipes* that completed third instar at different temperatures and photoperiod

Temperature(^{0}C)	Photoperiod L:D	Percentage of individuals that completed III Instar on different days													Total
		Dead 0	4	5	6	7	8	9	10	11	12	13	14	16	
25	12:12	15.00	0.00	0.00	0.00	0.00	0.00	10.00	20.00	25.00	5.00	5.00	20.00	0.00	100.00
	24:0	45.00	0.00	0.00	0.00	0.00	0.00	5.00	5.00	0.00	20.00	10.00	10.00	5.00	100.00
	0:24	21.62	0.00	0.00	0.00	0.00	18.92	29.73	16.22	10.81	2.70	0.00	0.00	0.00	100.00
30	12:12	0.00	0.00	22.45	26.53	0.00	36.73	0.00	14.29	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	0.00	0.00	9.52	16.67	4.76	38.10	2.38	28.57	0.00	0.00	0.00	0.00	0.00	100.00
	0:24	0.00	0.00	26.92	21.15	0.00	38.46	13.46	0.00	0.00	0.00	0.00	0.00	0.00	100.00
32.5	12:12	3.57	0.00	67.86	19.64	5.36	0.00	0.00	3.57	0.00	0.00	0.00	0.00	0.00	100.00
35	12:12	0.00	10.20	83.67	4.08	2.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 260.550, df=57, p=.000000

Table 5 -Percentage of individuals of *N.rufipes* that completed fourth instar at different temperatures and photoperiod

Temperature(^{0}C)	**Photoperiod L:D**	**Percentage of individuals that completed IV Instar on different days**															**Total**
		Dead 0	2	5	6	7	8	36	37	38	39	40	42	43	47	51	
25	12:12	76.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.76	5.88	0.00	5.88	0.00	0.00	0.00	100.00
	24:0	63.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	9.09	0.00	9.09	9.09	9.09	100.00
	0:24	75.86	0.00	0.00	0.00	0.00	0.00	13.79	6.90	0.00	0.00	3.45	0.00	0.00	0.00	0.00	100.00
30	12:12	0.00	0.00	14.29	53.06	16.33	16.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	0.00	4.76	4.76	33.33	23.81	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	0:24	0.00	0.00	14.29	53.06	16.33	16.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
32.5	12:12	0.00	0.00	18.52	59.26	22.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
35	12:12	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 327.301, df=72, p=0.00000

Table 6 -Percentage of individuals of *N.rufipes* that completed pre-pupal stage at different temperatures and photoperiod

Temperature(^{0}C)	**Photoperiod L:D**	**Percentage of individuals that completed pre-pupal stage on different days**									
		Dead 0	2	3	4	5	6	7	8	9	Total
30	12:12	0.00	0.00	0.00	0.00	0.00	48.98	32.65	18.37	0.00	100.00
	24:0	0.00	0.00	30.95	26.19	33.33	9.52	0.00	0.00	0.00	100.00
	0:24	0.00	0.00	0.00	0.00	36.54	28.85	21.15	13.46	0.00	100.00
32.5	12:12	0.00	3.70	68.52	25.93	1.85	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 462.040, df=27, p=0.00000

Table 7 - Percentage of individuals of *N.rufipes* that completed pupal stage at different temperatures and photoperiod

Temperature(^{0}C)	**Photoperiod L:D**	**Percentage of individuals that completed pupal stage on different days**										**Total**
		5	6	7	8	9	10	11	12	13	18	
30	12:12	0.00	0.00	0.00	16.33	0.00	8.16	16.33	48.98	8.16	2.04	100.00
	24:0	0.00	0.00	0.00	7.14	0.00	7.14	11.90	64.29	9.52	0.00	100.00
	0:24	0.00	0.00	21.15	17.31	26.92	0.00	25.00	9.62	0.00	0.00	100.00
32.5	12:12	12.96	87.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 78.2715, df=14, p=.000000

Table 8 - Influence of Incubation period (IP) on 1st instar duration of *N.rufipes* at 25 & 30^{0}C and different photoperiods (Pp)

T (^{0}C)	Pp L:D	Days in IP	Percentage of individuals that completed 1st Instar on different days																Total
			0	3	4	5	6	7	8	9	11	12	14	15	16	17	19	20-30	
25	0:24	2	15	12.5	0	22.5	20	5	0	10	2.5	0	2.5	2.5	2.5	0	2.5	2.5	100
		3	50	0	0	0	35.7	0	0	7.1	0	0	0	0	7.1	0	0	0	100
		4	50	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	100
	24:0	2	47.6	0	0	9.5	0	4.7	14.2	9.5	0	9.5	4.7	0	0	0	0	0	100
		3	57.8	0	0	0	5.2	10.5	5.2	0	0	0	5.2	0	0	5.2	0	10.4	100
		4	75	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	100
		5	50	0	12.5	0	0	0	12.5	25	0	0	0	0	0	0	0	0	100
	12:12	2	45.4	3.0	0	3.0	18.1	12.1	3.0	3.0	3.0	0	0	3.0	0	0	0	6.0	100
		3	36.8	5.2	5.2	21.0	5.2	10.5	0	5.2	0	0	0	0	0	5.2	0	5.2	100
30	0:24	2	4.5	0	15.9	79.5	0	0	0	0	0	0	0	0	0	0	0	0	100
		3	21.4	0	14.2	64.2	0	0	0	0	0	0	0	0	0	0	0	0	100
	24:0	2	13.1	0	0	10.5	65.7	10.5	0	0	0	0	0	0	0	0	0	0	100
		3	17.6	0	0	23.5	35.2	23.5	0	0	0	0	0	0	0	0	0	0	100
	12:12	2	8.5	0	0	21.2	70.2	0	0	0	0	0	0	0	0	0	0	0	100
		3	0	0	0	30	70	0	0	0	0	0	0	0	0	0	0	0	100

Pearson Chi-square: 920.991, df=477, p=.000000

Table 9 - Influence of Incubation period (IP) on 2nd instar duration of *N.rufipes* at 25 and 30^{0}C and different photoperiods (Pp)

Temperature (^{0}C)	Pp L:D	Days in IP	Duration of 2nd instar (in days)														Total
			0	4	5	6	7	8	9	10	11	12	13	14	15	17	
25	0:24	2	14.71	0.00	0.00	20.59	5.88	0.00	20.59	8.82	26.47	0.00	2.94	0.00	0.00	0.00	100.00
		3	0.00	0.00	0.00	28.57	0.00	0.00	14.29	14.29	42.86	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	2	0.00	0.00	0.00	0.00	0.00	9.09	0.00	36.36	0.00	0.00	9.09	0.00	27.27	18.18	100.00
		3	12.50	0.00	0.00	0.00	25.00	0.00	12.50	0.00	0.00	0.00	37.50	0.00	12.50	0.00	100.00
		5	75.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.00	0.00	100.00
	12:12	2	44.44	0.00	0.00	11.11	5.56	0.00	0.00	22.22	11.11	5.56	0.00	0.00	0.00	0.00	100.00
		3	25.00	0.00	0.00	0.00	0.00	8.33	0.00	0.00	50.00	8.33	0.00	8.33	0.00	0.00	100.00
30	0:24	2	2.38	7.14	4.76	30.95	30.95	23.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
		3	0.00	0.00	18.18	36.36	27.27	18.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	2	6.06	0.00	6.06	24.24	39.39	24.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
		3	21.43	0.00	7.14	21.43	21.43	28.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	12:12	2	6.98	2.33	6.98	32.56	27.91	23.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00
		3	10.00	0.00	20.00	50.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 897.762, df=316, p=0.00000

Table 10 - Influence of Incubation period (IP) on 3rd instar duration of *N.rufipes* at 25 and 30^{0}C and different photoperiods

Temperature(^{0}C)	Photoperiod L:D	Days in IP	Duration of 3rd instar (in days)												Total
			0	5	6	7	8	9	10	11	12	13	14	16	
25	**0:24**	2	20.69	0.00	0.00	0.00	13.79	34.48	20.69	6.90	3.45	0.00	0.00	0.00	100.00
		3	28.57	0.00	0.00	0.00	42.86	0.00	0.00	28.57	0.00	0.00	0.00	0.00	100.00
	24:0	2	36.36	0.00	0.00	0.00	0.00	0.00	9.09	0.00	18.18	18.18	18.18	0.00	100.00
		3	57.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	28.57	0.00	0.00	14.29	100.00
	12:12	2	20.00	0.00	0.00	0.00	0.00	20.00	20.00	10.00	0.00	0.00	30.00	0.00	100.00
		3	11.11	0.00	0.00	0.00	0.00	0.00	22.22	44.44	11.11	0.00	11.11	0.00	100.00
30	**0:24**	2	0.00	26.83	21.95	0.00	39.02	12.20	0.00	0.00	0.00	0.00	0.00	0.00	100.00
		3	0.00	27.27	18.18	0.00	36.36	18.18	0.00	0.00	0.00	0.00	0.00	0.00	100.00
	24:0	2	0.00	9.68	12.90	6.45	38.71	3.23	29.03	0.00	0.00	0.00	0.00	0.00	100.00
		3	0.00	9.09	27.27	0.00	36.36	0.00	27.27	0.00	0.00	0.00	0.00	0.00	100.00
	12:12	2	0.00	22.50	22.50	0.00	37.50	0.00	17.50	0.00	0.00	0.00	0.00	0.00	100.00
		3	0.00	22.22	44.44	0.00	33.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 863.148, df=270, p=0.00000

Table 11 - Influence of Incubation period on 4th instar duration of *N.rufipes* at 25 and 30^{0}C and different photoperiods

Temperature (^{0}C)	Photoperiod L:D	Days in IP	Duration of 4th instar (in days)														Total
			0	2	5	6	7	8	36	37	38	39	40	42	43	47	51	
25	0:24	2	69.5	0.00	0.00	0.00	0.00	0.00	17.3	8.70	0.00	0.00	4.35	0.00	0.00	0.00	0.00	100
	24:0	2	71.4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.29	0.00	14.29	0.00	0.00	100
		3	66.6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	33.33	0.00	100
	12:12	2	75.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00	100
		3	75.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	12.50	0.00	12.50	0.00	0.00	0.00	100
30	0:24	2	0.00	0.00	14.63	56.10	12.20	17.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
		3	0.00	0.00	18.18	45.45	18.18	18.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
	24:0	2	0.00	3.23	6.45	38.71	16.13	35.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
		3	0.00	9.09	0.00	18.18	45.45	27.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
	12:12	2	0.00	0.00	17.50	55.00	10.00	17.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100
		3	0.00	0.00	0.00	44.44	44.44	11.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100

Pearson Chi-square: 1878.65, df=339, p=0.00000

Table 12 - Influence of Incubation period (IP) on pre-pupal duration of *N.rufipes* at 25 and 30^0C and different photoperiods

Temperature (^{0}C)	Photoperiod L: D	Days I n IP	Duration of pre-pupa (in days)						Total
			0	5	6	7	8	9	
30	**0:24**	2	0.00	43.90	21.95	21.95	12.20	0.00	100.00
		3	0.00	9.09	54.55	18.18	18.18	0.00	100.00
	24:0	2	0.00	0.00	22.58	29.03	38.71	9.68	100.00
		3	0.00	0.00	54.55	18.18	18.18	9.09	100.00
	0:24	2	0.00	0.00	45.00	32.50	22.50	0.00	100.00
		3	0.00	0.00	66.67	33.33	0.00	0.00	100.00

Pearson Chi-square: 658.943, df=62, p=0.00000

Table 13 -Influence of Incubation period (IP) on 1st instar duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in IP	**Percentage of individuals that completed 1st Instar on different days**			Total
	0	4	5	
2	4.55	15.91	79.55	100.00
3	21.43	14.29	64.29	100.00

Pearson Chi-square: 3.85573, df=2, p=.145475

Table 14-Influence of Incubation period (IP) on 2nd instar duration of *N.rufipes* at 30^{0}C and photoperiod L:D 00:24

No. of days in IP	**Percentage of individuals that completed 2nd Instar on different days**						Total
	0	4	5	6	7	8	
2	2.38	7.14	4.76	30.95	30.95	23.81	100.00
3	0.00	0.00	18.18	36.36	27.27	18.18	100.00

Pearson Chi-square: 3.36831, df=5, p=.643403

Table 15- Influence of Incubation period (IP) on 3rd instar duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in IP	**Percentage of individuals that completed 3rd Instar on different days**				Total
	5	6	8	9	
2	26.83	21.95	39.02	12.20	100.00
3	27.27	18.18	36.36	18.18	100.00

Pearson Chi-square: .305768, df=3, p=.958939

Table 16 -Influence of Incubation period (IP) on 4th instar duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in IP	**Percentage of individuals that completed 4th Instar on different days**				Total
	5	6	7	8	
2	14.63	56.10	12.20	17.07	100.00
3	18.18	45.45	18.18	18.18	100.00

Pearson Chi-square: .490480, df=3, p=.920977

Table 17 -Influence of Incubation period (IP) on pre-pupal duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in IP	**Percentage of individuals that completed pre-pupal stage on different days**				Total
	5	6	7	8	
2	43.90	21.95	21.95	12.20	100.00
3	9.09	54.55	18.18	18.18	100.00

Pearson Chi-square: 6.35994, df=3, p=.095375

Table 18 - Influence of Incubation period (IP) on pupal duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in IP	**Percentage of individuals that completed pupal stage on different days**					Total
	7	8	9	11	12	
2	21.95	19.51	24.39	26.83	7.32	100.00
3	18.18	9.09	36.36	18.18	18.18	100.00

Pearson Chi-square: 2.38848, df=4, p=.664713

Table 19 -Influence of time spent in 1st instar on 2nd instar duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 1st instar	**Percentage of individuals that completed 2nd Instar on different days**						**Total**
	0 (dead)	**4**	**5**	**6**	**7**	**8**	
4	0.00	0.00	0.00	77.78	0.00	22.22	100.00
5	2.27	6.82	9.09	22.73	36.36	22.73	100.00

Pearson Chi-square: 11.9694, df=5, p=.035233

Table 20 -Influence of time spent in1st instar on 3rd instar duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 1st instar	**Percentage of individuals that completed 3rd Instar on different days**				**Total**
	5	6	8	9	
4	22.22	11.11	55.56	11.11	100.00
5	27.91	23.26	34.88	13.95	100.00

Pearson Chi-square: 1.47978, df=3, p=.686947

Table 21 -Influence of time spent in1st instar on 4th instar duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 1st instar	**Percentage of individuals that completed 4th Instar on different days**				**Total**
	5	6	7	8	
4	0.00	33.33	22.22	44.44	100.00
5	18.60	58.14	11.63	11.63	100.00

Pearson Chi-square: 7.77622, df=3, p=.050889

Table 22 -Influence of time spent in 1st instar on pre-pupal duration of *N.rufipes* at 30^0C and photoperiod L:D 00:24

No. of days in 1st instar	**Percentage of individuals that completed pre-pupal stage on different days**				**Total**
	5	6	7	8	
4	44.44	22.22	22.22	11.11	100.00
5	34.88	30.23	20.93	13.95	100.00

Pearson Chi-square: .402268, df=3, p=.939773

Table 23- Influence of time spent in 1st instar on pupal duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 1st instar	**Percentage of individuals that completed pupal stage on different days**					**Total**
	7	8	9	11	12	
4	33.33	22.22	33.33	0.00	11.11	100.00
5	18.60	16.28	25.58	30.23	9.30	100.00

Pearson Chi-square: 3.82746, df=4, p=.429875

Table 24 -Influence of time spent in 2nd instar on 3rd instar duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 2nd instar	**Percentage of individuals that completed 3rd Instar on different days**				**Total**
	5	6	8	9	
4	66.67	0.00	33.33	0.00	100.00
5	50.00	50.00	0.00	0.00	100.00
6	29.41	17.65	35.29	17.65	100.00
7	12.50	37.50	37.50	12.50	100.00
8	25.00	0.00	58.33	16.67	100.00

Pearson Chi-square: 14.8148, df=12, p=.251786

Table 25-Influence of time spent in 2nd instar on 4th instar duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 2nd instar	**Percentage of individuals that completed 4th Instar on different days**				**Total**
	5	6	7	8	
4	33.33	66.67	0.00	0.00	100.00
5	50.00	25.00	25.00	0.00	100.00
6	5.88	52.94	11.76	29.41	100.00
7	12.50	56.25	12.50	18.75	100.00
8	16.67	58.33	16.67	8.33	100.00

Pearson Chi-square: 9.78161, df=12, p=.635110

Table 26 -Influence of time spent in 2nd instar on pre-pupal duration of *N.rufipes* at 30^0C and photoperiod L: D 00: 24

No. of days in 2nd instar	**Percentage of individuals that completed pre-pupal stage on different days**				**Total**
	5	6	7	8	
4	0.00	33.33	33.33	33.33	100.00
5	0.00	25.00	75.00	0.00	100.00
6	41.18	17.65	17.65	23.53	100.00
7	31.25	50.00	12.50	6.25	100.00
8	58.33	16.67	16.67	8.33	100.00

Pearson Chi-square: 18.2435, df=12, p=.108561

Table 27 - Influence of time spent in 2nd instar on pupal duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in 2nd instar	**Percentage of individuals that completed pupal stage on different days**					**Total**
	7	8	9	11	12	
4	33.33	33.33	0.00	33.33	0.00	100.00
5	25.00	25.00	25.00	0.00	25.00	100.00
6	11.76	17.65	41.18	29.41	0.00	100.00
7	31.25	25.00	0.00	25.00	18.75	100.00
8	16.67	0.00	50.00	25.00	8.33	100.00

Pearson Chi-square: 19.3486, df=16, p=.251079

Table 28 -Influence of time spent in 3rd instar on 4th instar duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in 3rd instar	**Percentage of individuals that completed 4th Instar on different days**				**Total**
	5	6	7	8	
5	21.43	50.00	21.43	7.14	100.00
6	18.18	63.64	9.09	9.09	100.00
8	15.00	45.00	10.00	30.00	100.00
9	0.00	71.43	14.29	14.29	100.00

Pearson Chi-square: 6.55514, df=9, p=.683321

Table 29 -Influence of time spent in 3rd instar on pre-pupal duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in 3rd instar	**Percentage of individuals that completed pre-pupal stage on different days**				**Total**
	5	6	7	8	
5	28.57	21.43	35.71	14.29	100.00
6	45.45	18.18	27.27	9.09	100.00
8	40.00	30.00	15.00	15.00	100.00
9	28.57	57.14	0.00	14.29	100.00

Pearson Chi-square: 6.96116, df=9, p=.641163

Table 30 -Influence of time spent in 3rd instar on pupal duration of *N.rufipes* at 30^{0}C and photoperiod L: D 00:24

No. of days in 3rd instar	**Percentage of individuals that completed pupal stage on different days**					**Total**
	7	**8**	**9**	**11**	**12**	
5	14.29	14.29	28.57	35.71	7.14	100.00
6	18.18	36.36	27.27	9.09	9.09	100.00
8	30.00	10.00	25.00	30.00	5.00	100.00
9	14.29	14.29	28.57	14.29	28.57	100.00

Pearson Chi-square: 9.76812, df=12, p=.636292

Table 31 -Influence of time spent in 4th instar on pre-pupal duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 4th instar	Percentage of individuals that completed pre-pupal stage on different days				Total
	5	6	7	8	
5	12.50	37.50	37.50	12.50	100.00
6	35.71	28.57	17.86	17.86	100.00
7	57.14	14.29	28.57	0.00	100.00
8	44.44	33.33	11.11	11.11	100.00

Pearson Chi-square: 6.17553, df=9, p=.722223

Table 32 - Influence of time spent in 4th instar on pupal duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in 4th instar	Percentage of individuals that completed pupal stage on different days					Total
	7	8	9	11	12	
5	25.00	0.00	12.50	50.00	12.50	100.00
6	21.43	21.43	28.57	25.00	3.57	100.00
7	14.29	0.00	42.86	0.00	42.86	100.00
8	22.22	33.33	22.22	22.22	0.00	100.00

Pearson Chi-square: 19.6253, df=12, p=.074576

Table 33 -Influence of time spent in pre-pupal stage on pupal duration of *N.rufipes* at 30^0C and photoperiod L: D 00:24

No. of days in Pre-pupal stage	Percentage of individuals that completed pupal stage on different days					Total
	7	8	9	11	12	
5	15.79	10.53	47.37	10.53	15.79	100.00
6	33.33	13.33	6.67	33.33	13.33	100.00
7	27.27	27.27	36.36	9.09	0.00	100.00
8	0.00	28.57	0.00	71.43	0.00	100.00

Pearson Chi-square: 24.1143, df=12, p=.019657

Table 34 - Influence of incubation period (IP) on1st instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in IP	Percentage of individuals that completed 1st instar on different days			Total
	0	3	4	
2	1.92	88.46	9.62	100.00
3	0.00	75.00	25.00	100.00

Pearson Chi-square: 1.70541, df=2, p=.426270

Table 35 - Influence of incubation period (IP) on 2nd instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in IP	Percentage of individuals that completed 2nd instar on different days						Total
	0	4	5	6	7	8	
2	5.88	11.76	11.76	35.29	33.33	1.96	100.00
3	0.00	0.00	12.50	50.00	37.50	0.00	100.00

Pearson Chi-square: 2.00826, df=5, p=.847996

Table 36 - Influence of incubation period (IP) on 3rd instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L:D 12:12

No. of days in IP	Percentage of individuals that completed 3rd Instar on different days					Total
	0	5	6	7	10	
2	4.17	68.75	16.67	6.25	4.17	100.00
3	0.00	62.50	37.50	0.00	0.00	100.00

Pearson Chi-square: 2.72129, df=4, p=.605499

Table 37 - Influence of incubation period (IP) on 4th instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in IP	Percentage of individuals that completed 4th Instar on different days			Total
	5	6	7	
2	17.39	60.87	21.74	100.00
3	25.00	50.00	25.00	100.00

Pearson Chi-square: .381522, df=2, p=.826331

Table 38 - Influence of incubation period (IP) on pre-pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in IP	Percentage of individuals that completed pre-pupal stage on different days				Total
	2	3	4	5	
2	0.00	76.09	21.74	2.17	100.00
3	25.00	25.00	50.00	0.00	100.00

Pearson Chi-square: 16.3691, df=3, p=.000954

Table 39 - Influence of incubation period (IP) on pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in IP	Percentage of individuals that completed pupal stage on different days		Total
	5	6	
2	10.87	89.13	100.00
3	25.00	75.00	100.00

Pearson Chi-square: 1.20603, df=1, p=.272129

Table 40 - Influence of time spent in 1st instar on 2nd instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 1st instar	Percentage of individuals that completed 2nd instar on different days						Total
	0	4	5	6	7	8	
3	3.85	9.62	7.69	42.31	34.62	1.92	100.00
4	14.29	14.29	42.86	0.00	28.57	0.00	100.00

Pearson Chi-square: 11.0474, df=5, p=.050474

Table 41 - Influence of time spent in 1st instar on 3rd instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 1st instar	Percentage of individuals that completed 3rd instar on different days					Total
	0	5	6	7	10	
3	4.00	70.00	16.00	6.00	4.00	100.00
4	0.00	50.00	50.00	0.00	0.00	100.00

Pearson Chi-square: 4.30852, df=4, p=.365879

Table 42 - Influence of time spent in 1st instar on 4th instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 1st instar	Percentage of individuals that completed 4th instar on different days			Total
	5	6	7	
3	16.67	60.42	22.92	100.00
4	33.33	50.00	16.67	100.00

Pearson Chi-square: .991406, df=2, p=.609147

Table 43 - Influence of time spent in 1st instar on pre-pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 1st instar	Percentage of individuals that completed pre-pupal stage on different days				Total
	2	3	4	5	
3	2.08	68.75	29.17	0.00	100.00
4	16.67	66.67	0.00	16.67	100.00

Pearson Chi-square: 12.8159, df=3, p=.005058

Table 44- Influence of time spent in 1st instar on pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L:D 12:12

No. of days in 1st instar	Percentage of individuals that completed pupal stage on different days		Total
	5	6	
3	12.50	87.50	100.00
4	16.67	83.33	100.00

Pearson Chi-square: .082067, df=1, p=.774518

Table 45 -Influence of time spent in 2nd instar on 3rd instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 2nd instar	Percentage of individuals that completed 3rd instar on different days					Total
	0	5	6	7	10	
4	16.67	33.33	16.67	33.33	0.00	100.00
5	14.29	71.43	14.29	0.00	0.00	100.00
6	0.00	63.64	22.73	4.55	9.09	100.00
7	0.00	80.00	20.00	0.00	0.00	100.00
8	0.00	100.00	0.00	0.00	0.00	100.00

Pearson Chi-square: 22.1958, df=16, p=.137071

Table 46 - Influence of time spent in 2nd instar on 3rd instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 2nd instar	Percentage of individuals that completed 4th instar on different days			Total
	5	6	7	
4	40.00	40.00	20.00	100.00
5	33.33	33.33	33.33	100.00
6	9.09	68.18	22.73	100.00
7	20.00	65.00	15.00	100.00
8	0.00	0.00	100.00	100.00

Pearson Chi-square: 8.75335, df=8, p=.363561

Table 47 - Influence of time spent in 2nd instar on pre-pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 2nd instar	Percentage of individuals that completed pre-pupal stage on different days				Total
	2	3	4	5	
4	0.00	100.00	0.00	0.00	100.00
5	16.67	83.33	0.00	0.00	100.00
6	4.55	63.64	31.82	0.00	100.00
7	0.00	60.00	35.00	5.00	100.00
8	0.00	100.00	0.00	0.00	100.00

Pearson Chi-square: 10.8166, df=12, p=.544711

Table 48 - Influence of time spent in 2nd instar on pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 2nd instar	Percentage of individuals that completed pupal stage on different days		Total
	5	6	
4	0.00	100.00	100.00
5	33.33	66.67	100.00
6	13.64	86.36	100.00
7	10.00	90.00	100.00
8	0.00	100.00	100.00

Pearson Chi-square: 3.26477, df=4, p=.514544

Table 49 - Influence of time spent in 3rd instar on 4th instar duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 3rd instar	Percentage of individuals that completed 4th instar on different days			Total
	5	6	7	
5	13.16	60.53	26.32	100.00
6	36.36	54.55	9.09	100.00
7	33.33	66.67	0.00	100.00
10	0.00	50.00	50.00	100.00

Pearson Chi-square: 5.81663, df=6, p=.444061

Table 50 - Influence of time spent in 3rd instar on pre-pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 3rd instar	Percentage of individuals that completed pre-pupal stage on different days				Total
	2	3	4	5	
5	2.63	68.42	28.95	0.00	100.00
6	9.09	54.55	27.27	9.09	100.00
7	0.00	100.00	0.00	0.00	100.00
10	0.00	100.00	0.00	0.00	100.00

Pearson Chi-square: 7.54869, df=9, p=.580193

Table 51- Influence of time spent in 3rd instar on pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 3rd instar	Percentage of individuals that completed pupal stage on different days		Total
	5	6	
5	10.53	89.47	100.00
6	18.18	81.82	100.00
7	0.00	100.00	100.00
10	50.00	50.00	100.00

Pearson Chi-square: 3.34393, df=3, p=.341592

Table 52- Influence of time spent in 4th instar on pre-pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 4th instar	Percentage of individuals that completed pre-pupal stage on different days				Total
	2	3	4	5	
5	20.00	50.00	30.00	0.00	100.00
6	0.00	68.75	28.13	3.13	100.00
7	0.00	83.33	16.67	0.00	100.00

Pearson Chi-square: 10.8932, df=6, p=.091772

Table 53- Influence of time spent in 4th instar on pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in 4th instar	Percentage of individuals that completed pupal stage on different days		Total
	5	6	
5	20.00	80.00	100.00
6	6.25	93.75	100.00
7	25.00	75.00	100.00

Pearson Chi-square: 3.25805, df=2, p=.196136

Table 54 - Influence of time spent in pre-pupal stage on pupal duration of *N.rufipes* at 32.5^{0}C and photoperiod L: D 12:12

No. of days in pre-pupal stage	Percentage of individuals that completed pupal stage on different days		Total
	5	6	
2	100.00	0.00	100.00
3	13.51	86.49	100.00
4	0.00	100.00	100.00
5	0.00	100.00	100.00

Pearson Chi-square: 15.6726, df=3, p=.001326

Table 55 -Mean longevity (in days) of male *N. rufipes* at different temperatures and photoperiods

Temperature (^{0}C)	Photoperiod L:D	Number	Mean (in days)	SD	p-level
	12:12	70	70.0000	24.15889	0.00000
	0:24	70	80.0429	27.12261	
30	24:0	70	58.6143	19.83723	
35	12:12	70	41.6000	20.23084	
	12:12	70	54.4857	21.20688	
	0:24	70	57.5286	21.28463	
25	24:0	70	46.7714	17.39163	
32.5	12:12	70	83.3429	30.27406	

Table 56 -ANOVA table showing significant difference between longevity of males of *N. rufipes* at different temperatures and photoperiod

Temperature (^{0}C)	Photoperiod L:D	30, 12:12 (1)	30,0:24 (2)	30,24:0 (3)	35,12:12 (4)	25,12:12 (5)	25,0:24 (6)	25,24:0 (7)	32.5,12:12 (8)
30 (1)	12:12		0.4668014	0.2885193	0.0000000	0.0277317	0.1767849	0.0000130	0.1117511
30 (2)	0:24	0.4668014		0.0001112	0.0000000	0.0000006	0.0000312	0.0000000	0.9981669
30 (3)	24:0	0.2885193	0.0001112		0.0086329	0.9924803	0.9999990	0.2373568	0.0000019
35 (4)	12:12	0.0000000	0.0000000	0.0086329		0.1431857	0.0204235	0.9714841	0.0000000
25 (5)	12:12	0.0277317	0.0000006	0.9924803	0.1431857		0.9989168	0.7877873	0.0000000
25 (6)	0:24	0.1767849	0.0000312	0.9999990	0.0204235	0.9989168		0.3676073	0.0000004
25 (7)	24:0	0.0000130	0.0000000	0.2373568	0.9714841	0.7877873	0.3676073		0.0000000
32.5 (8)	12:12	0.1117511	0.9981669	0.0000019	0.0000000	0.0000000	0.0000004	0.0000000	

Figure 2 - Box plot showing mean longevity (in days) of male *N. rufipes* at different temperatures and photoperiods

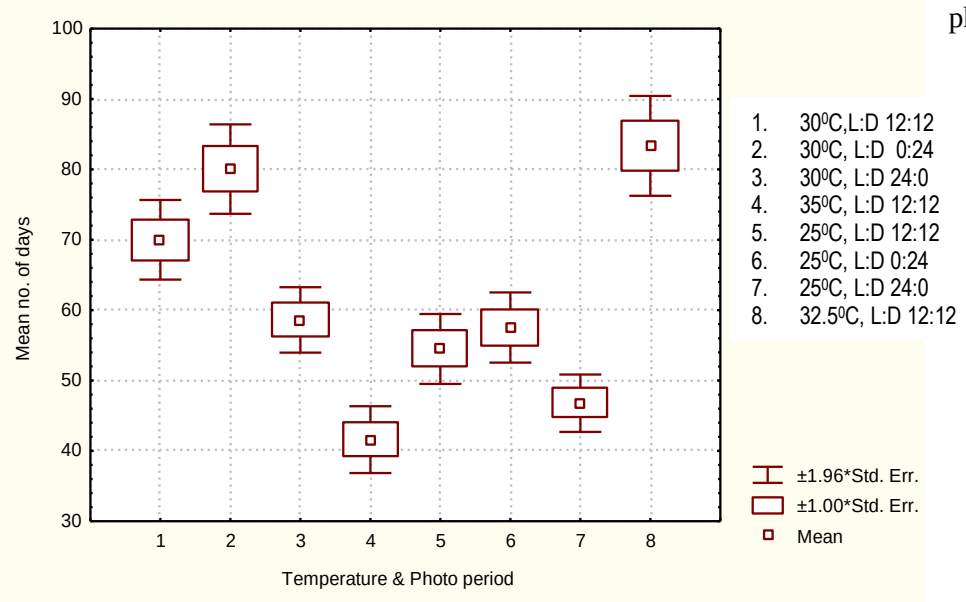

Table 57- Mean longevity (in days) of female *N. rufipes* at different temperatures and photoperiod

Temperature (^{0}C)	Photoperiod L:D	Number	Mean (in days)	SD	p-level
30	12:12	70	79.15714	21.20544	0.0000
30	0:24	70	93.62857	28.02699	
30	24:0	70	67.78571	18.16103	
35	12:12	70	48.18571	17.87176	
25	12:12	70	62.48571	18.40663	
25	0:24	70	66.47143	19.46958	
25	24:0	70	55.64286	17.08549	
32.5	12:12	70	95.58572	31.59274	

Table 58 -ANOVA table showing significant difference between longevity of females of *N. rufipes* at different temperatures and photoperiod

Temperature (^{0}C)	Photoperiod L:D	30, 12:12 (1)	30,0:24 (2)	30,24:0 (3)	35,12:12 (4)	25,12:12 (5)	25,0:24 (6)	25,24:0 (7)	32.5,12:12 (8)
30 (1)	12:12		0.0369022	0.2340983	0.0000000	0.0061859	0.1179194	0.0000023	0.0076768
30 (2)	0:24	0.0369022		0.0000001	0.0000000	0.0000000	0.0000000	0.0000000	0.9999239
30 (3)	24:0	0.2340983	0.0000001		0.0003214	0.9583792	0.9999951	0.1592973	0.0000000
35 (4)	12:12	0.0000000	0.0000000	0.0003214		0.0417271	0.0013123	0.7793235	0.0000000
25 (5)	12:12	0.0061859	0.0000000	0.9583792	0.0417271		0.9921014	0.8484495	0.0000000
25 (6)	0:24	0.1179194	0.0000000	0.9999951	0.0013123	0.9921014		0.2978957	0.0000000
25 (7)	24:0	0.0000023	0.0000000	0.1592973	0.7793235	0.8484495	0.2978957		0.0000000
32.5 (8)	12:12	0.0076768	0.9999239	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	

Figure 3- Box plot showing mean longevity (in days) of females of *N. rufipes* at different temperatures and photoperiods

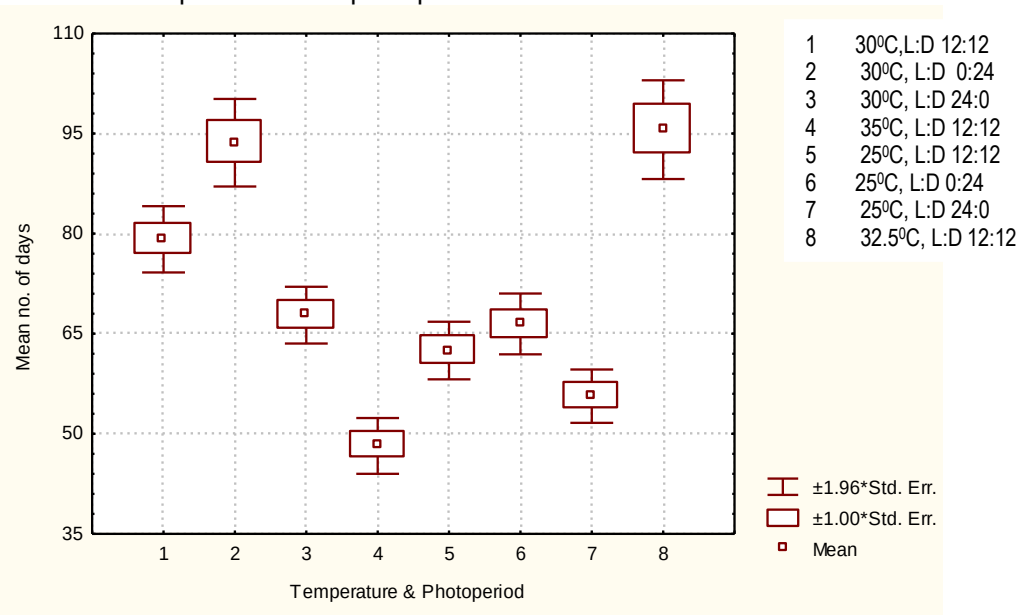

Figure 4 - Mean longevity (in days) of male and female *N. rufipes* at different temperatures and photoperiod

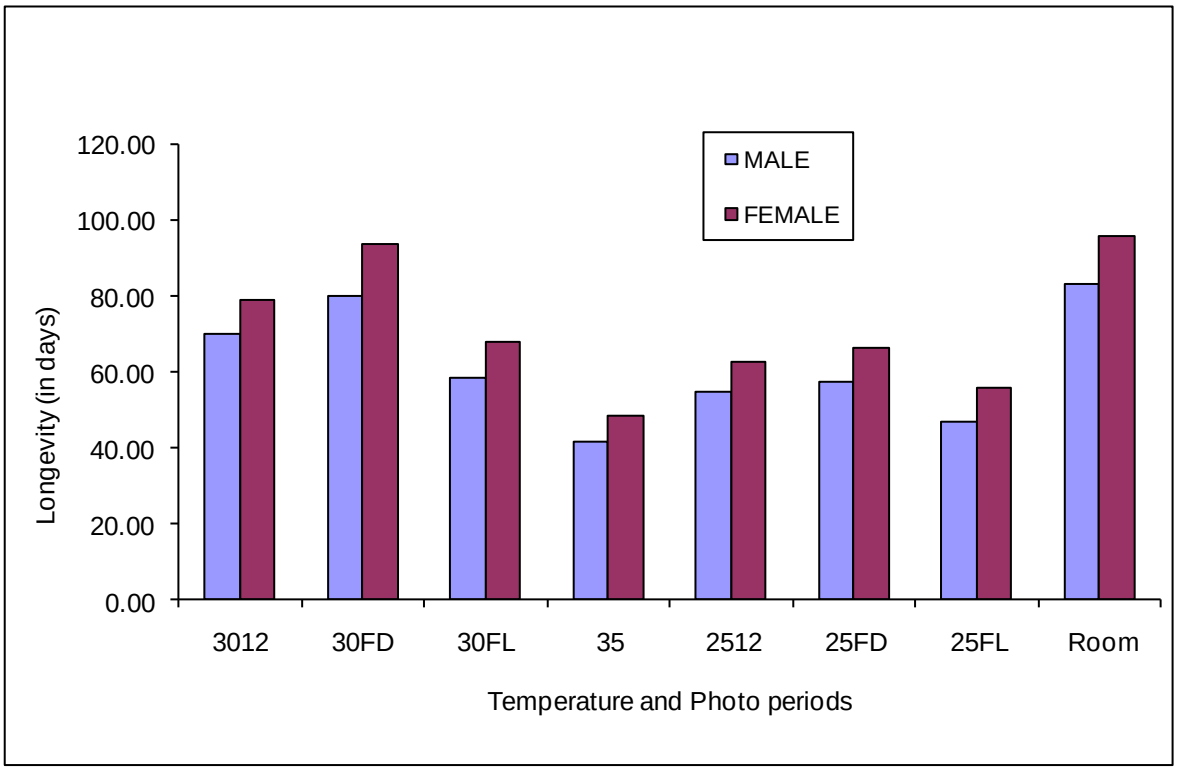

Table 59 -Mean longevity (in days) of male and female *N. rufipes* at 30^0C and L: D 12:12

	Number	Mean (in days)	SD	p-level
Male	70	70.0000	24.1589	0.018517
Female	70	79.1571	21.2054	

Figure 5 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 30^0C and L: D 12:12

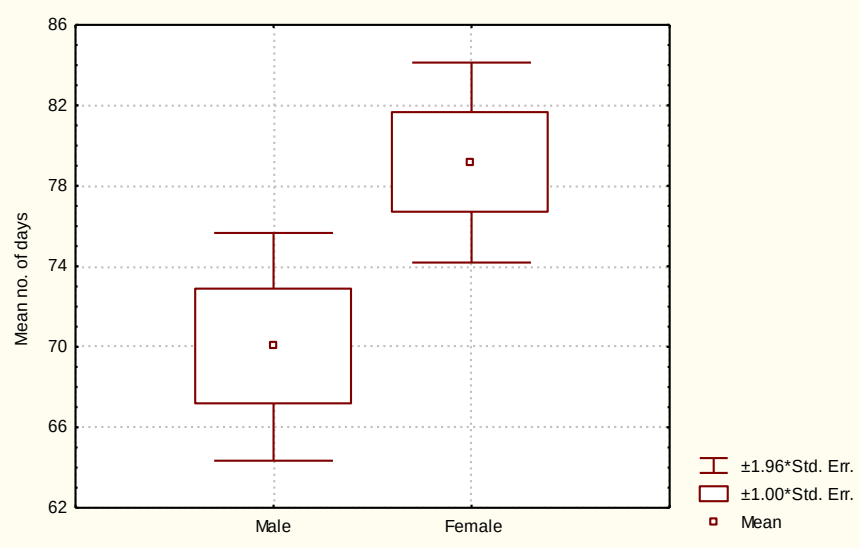

Table 60 -Mean longevity (in days) of male and female *N. rufipes* at 30^0C and L: D 0:24

	Number	Mean (in days)	SD	p-level
Male	70	80.04285	27.12261	0.00416
Female	70	93.62857	28.02699	

Figure 7 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 30^0C and L: D 0:24

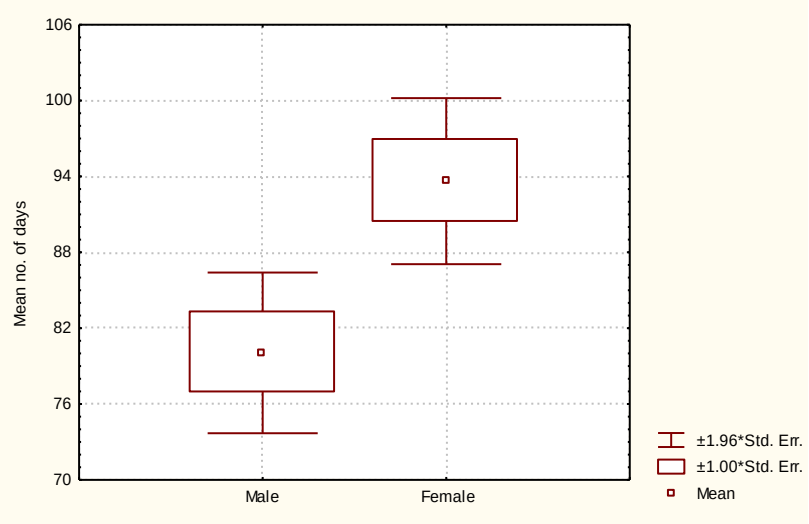

Table 61 -Mean longevity (in days) of male and female *N. rufipes* at 30^0C and L: D 24:0

	Number	Mean (in days)	SD	p-level
Male	70	58.61428	19.83723	0.004997
Female	70	67.78571	18.16103	

Figure 7 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 30^0C and L: D 24:0

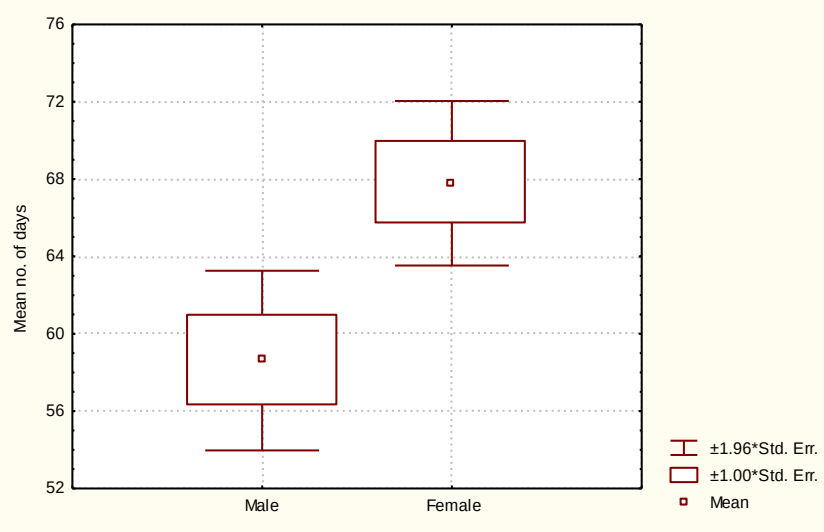

Table 62 -Mean longevity (in days) of male and female *N. rufipes* at 35^0C and L: D 12:12

	Number	Mean (in days)	SD	p-level
Male	70	41.6	20.23084	0.043139
Female	70	48.18571	17.87176	

Figure 8 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 35^0C and L: D 12:12

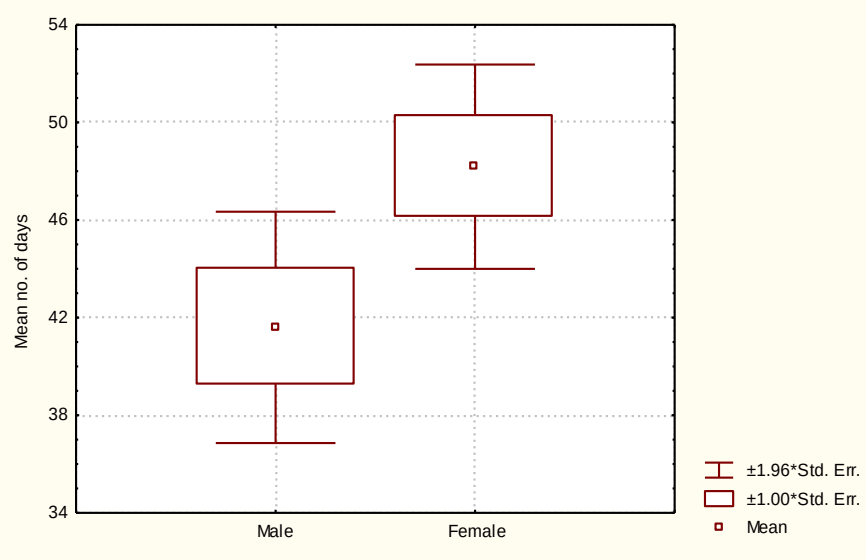

Table 63 -Mean longevity (in days) of male and female *N. rufipes* at 25^0C and L: D 12:12

	Number	Mean	SD	p-level
Male	70	54.48571	21.20688	0.018507
Female	70	62.48571	18.40663	

Figure 9 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 25^0C and L: D 12:12

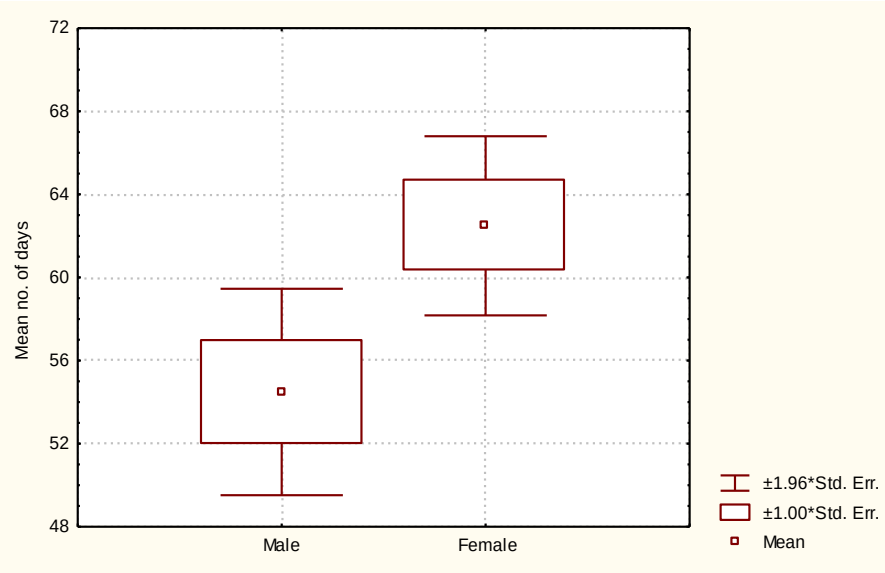

Table 64 -Mean longevity (in days) of male and female *N. rufipes* at 25^0C and L: D 0:24

	Number	Mean (in days)	SD	p-level
Male	70	57.52857	21.28463	0.010516
Female	70	66.47143	19.46958	

Figure 10 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 25^0C and L: D 0:24

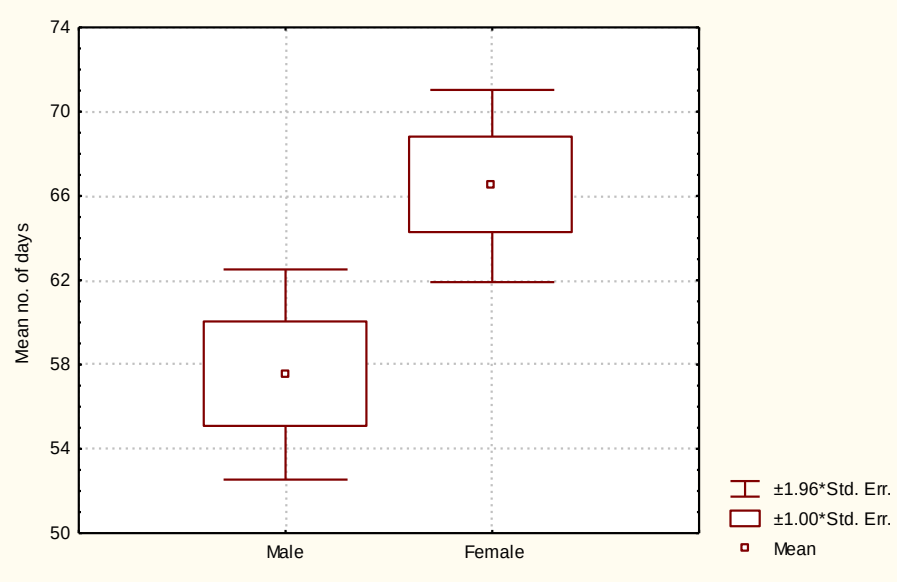

Table 65 -Mean longevity (in days) of male and female *N. rufipes* at 25^0C and L: D 24:0

	Number	Mean (in days)	SD	p-level
Male	70	46.77143	17.39163	0.002792
Female	70	55.64286	17.08549	

Figure 11 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 25^0C and L: D 24:0

Table 66 -Mean longevity (in days) of male and female *N. rufipes* at 32.5^{0}C and L: D 12:12

	Number	Mean (in days)	SD	p-level
Male	70	83.34286	30.27406	0.020667
Female	70	95.58572	31.59274	

Figure 12 -Box plot showing mean longevity (in days) of male and female *N. rufipes* at 32.5^{0}C and L: D 12:12

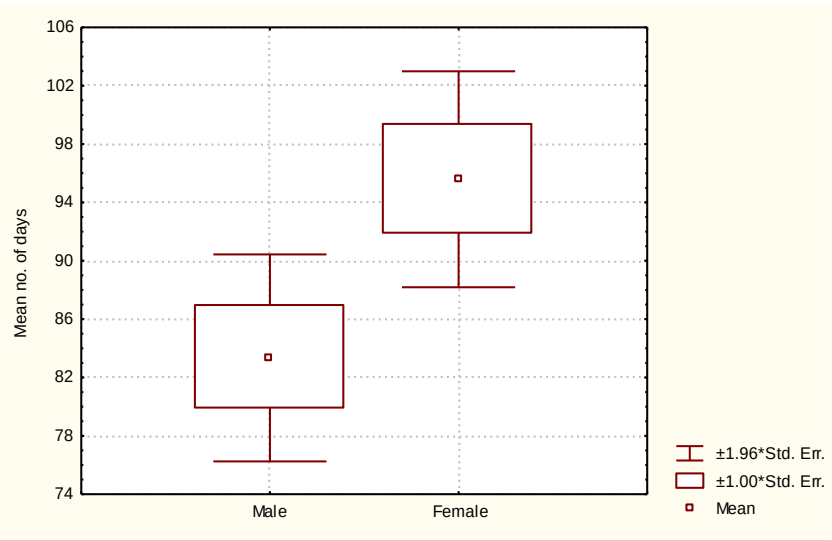

Table 67 -Mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25 & 30^{0}C at L:D 0:24

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	0:24	20	34.6000	6.3776	0.00000000
30		20	90.6500	27.9704	

Figure 13 - Box plot showing mean number of eggs laid after double mating of freshly mated females at 25 & 30^{0}C at L:D 0:24

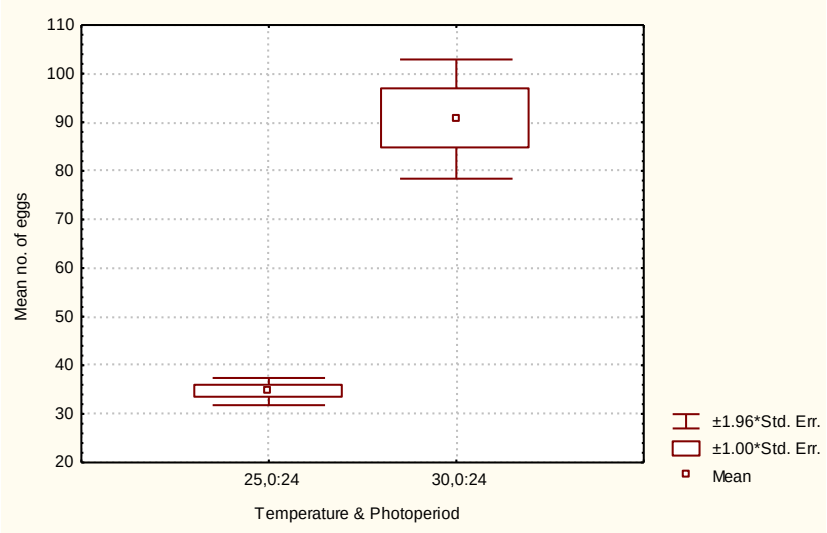

Table 68 -Mean number of eggs laid after multiple mating of freshly mated females at 25 & 30^{0}C at L:D 0:24

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	0:24	20	52.7500	8.2836	0.00000000
30		20	158.1000	25.9816	

Figure 14 - Box plot showing mean number of eggs laid after multiple mating of freshly mated females at 25 & 30^{0}C at L:D 0:24

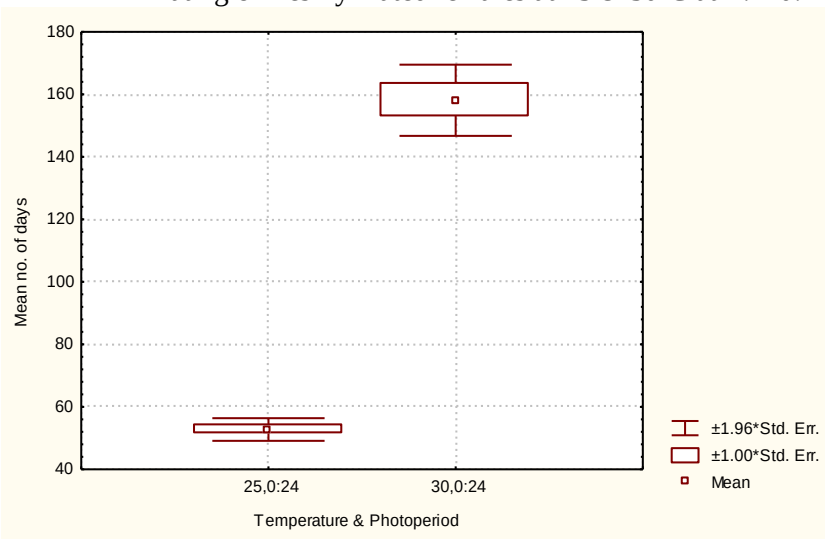

Table 69-Mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25 & 30^{0}C at L:D 24:0

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	24:0	20	17.5000	3.0000	0.00000000
30		20	53.4000	14.6014	

Figure 15 - Box plot showing mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25 & 30^{0}C at L: D 24:0

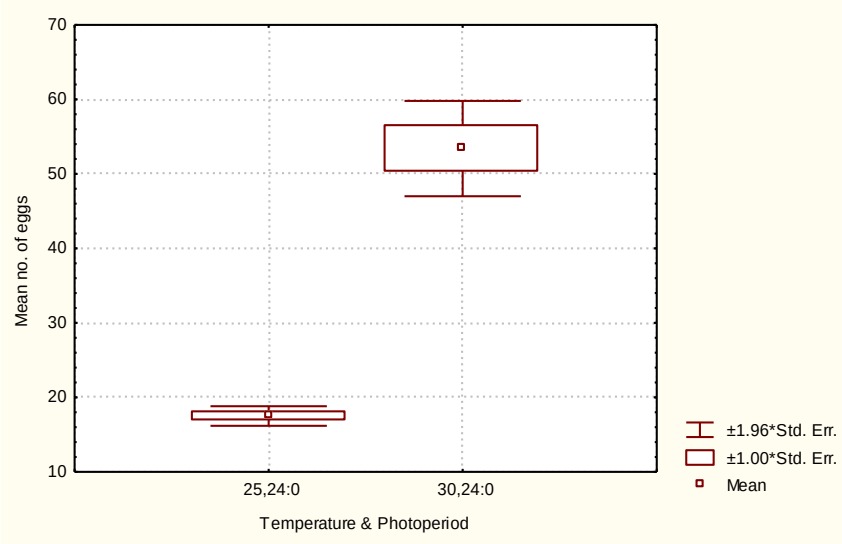

Table 70 -Mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25 & 30^{0}C at L:D 24:0

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	24:0	20	25.4000	7.1333	0.00000000
30		20	99.1000	17.6930	

Figure 16 - Box plot showing mean number of eggs laid after multiple mating of freshly mated females at 25 & 30^{0}C at L: D 24:0

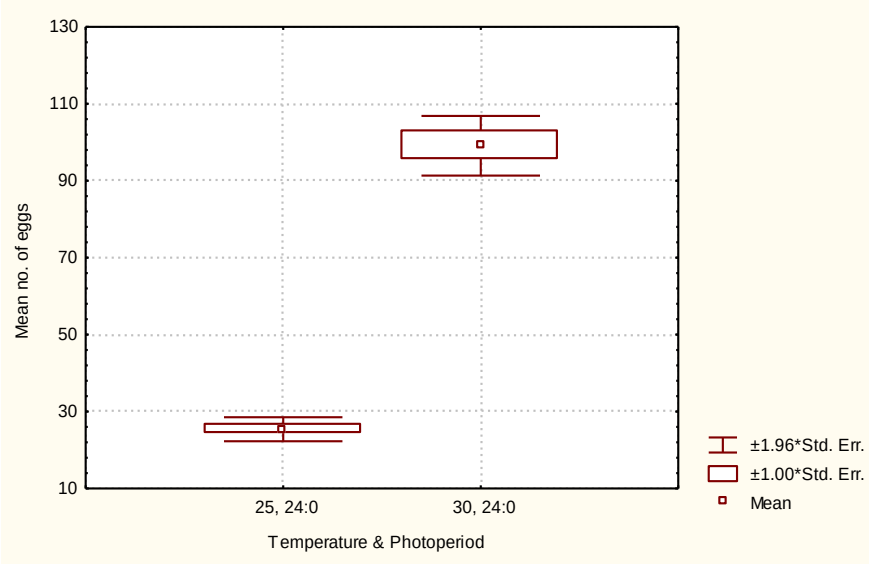

Table 71- Mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25 and 30^{0}C and photoperiod L:D 12:12

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	12:12	20	22	4.576829	
30		20	71	23.86585	**5.57E-11**

Figure 17 - Box plot showing mean number of eggs laid after double mating of freshly mated females at 25 and 30^{0}C and photoperiod L:D 12:12

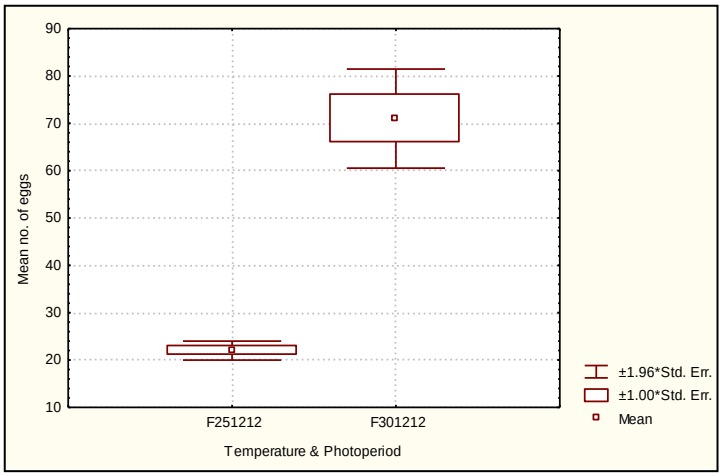

Table 72 -Mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25and 30^{0}C and photoperiod L:D 12:12

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	12:12	20	39.55	12.93903	
30		20	122.15	24.29484	**5.37E-16**

Figure 18 - Box plot showing mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25 and 30^{0}C and photoperiod L:D 12:12

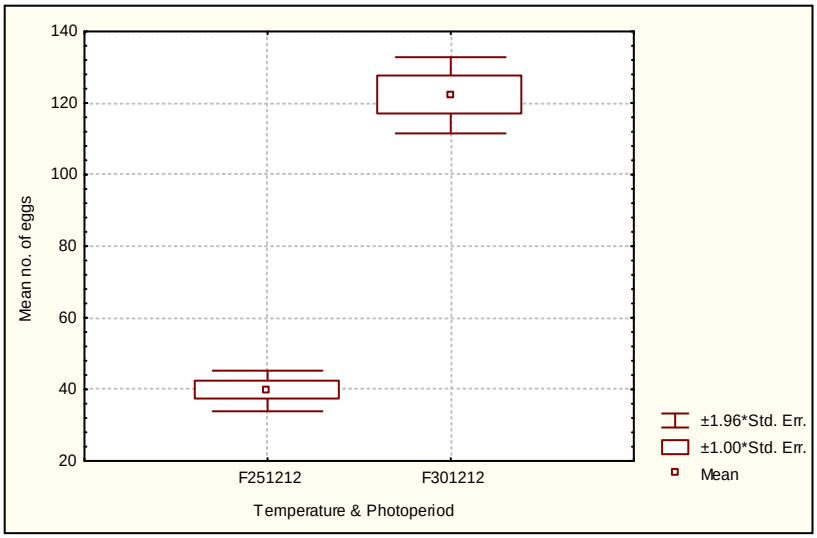

Table 73 -Mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25^{0}C and different photoperiods

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	12:12	20	22.0000	4.5768	**1.16E-15**
	0:24	20	34.6000	6.3776	
	24:0	20	17.5000	3.0000	

Figure 19 - Box plot showing mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25^{0}C and different photoperiods

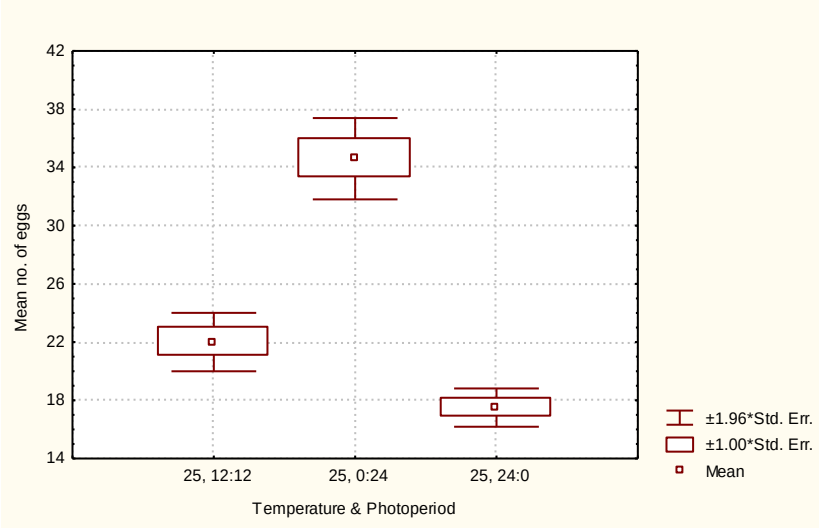

Table 74-Mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25^{0}C and different photoperiods

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
25	12:12	20	39.5500	12.9390	**2.02E-11**
	0:24	20	52.7500	8.2836	
	24:0	20	25.4000	7.1333	

Figure 20- Box plot showing mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25^{0}C and different photoperiods

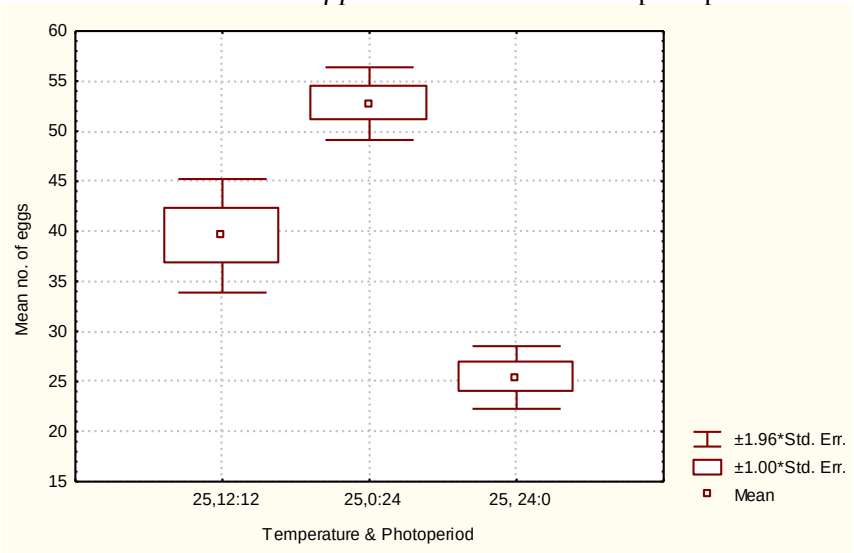

Table 75 - Mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 30^{0}C and different photoperiods

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
30	12:12	20	71	23.86585	**1.8E-05**
	0:24	20	90.6500	27.9704	
	24:0	20	53.4000	14.6014	

Figure 21- Box plot showing mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 30^{0}C and different photoperiod

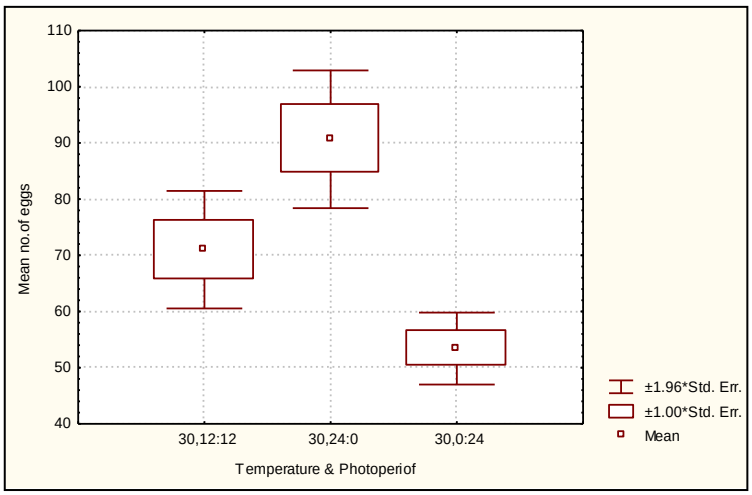

Table 76-Mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 30^{0}C and different photoperiods

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
30	12:12	20	122.15	24.29484	**2.28E-10**
	0:24	20	158.1	25.98157	
	24:0	20	99.1	17.693	

Figure 22 - Box plot showing mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 30^{0}C and different photoperiod

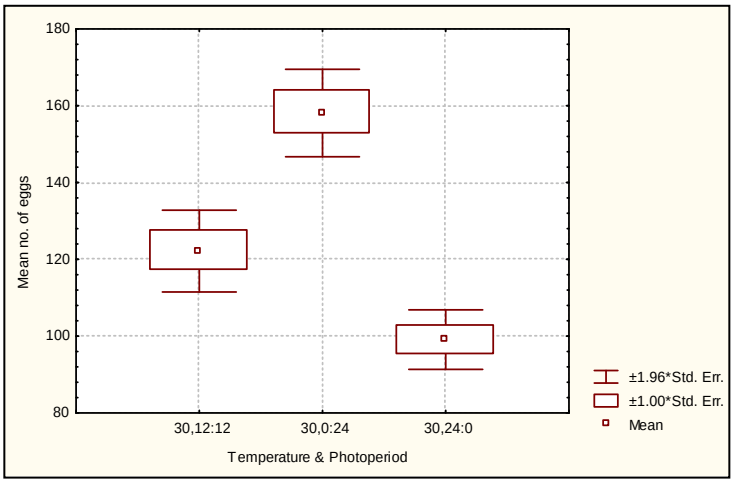

Table 77- Mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25 and 30 and 32.5^{0}C and photoperiod L:D 12:12

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
32.5	12:12	20	148.4	20.76029	1.56E-23
25		20	22	4.576829	
30		20	90.45	33.67878	

Figure 23 - Box plot showing mean number of eggs laid after double mating of freshly mated females of *N. rufipes* at 25 and 30 and 32.5^{0}C and photoperiod L:D 12:12

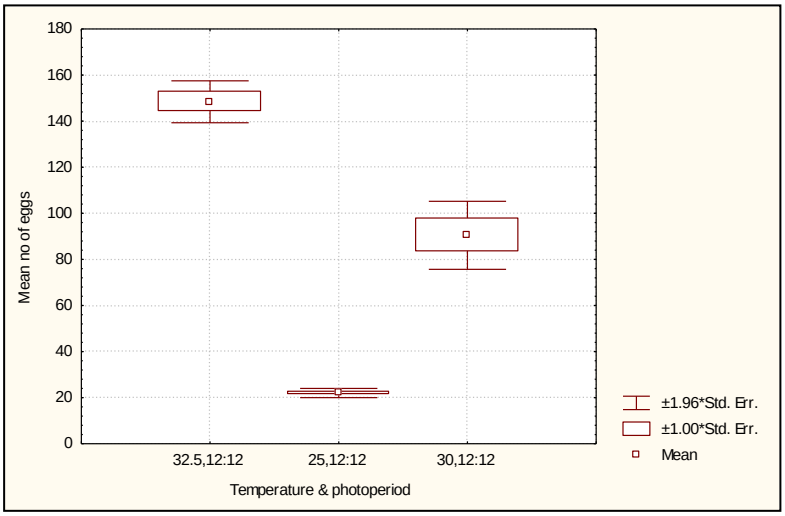

Table 78 -Mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25, 30 and 32.5^{0}C and photoperiod L:D 12:12

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
32.5	12:12	20	227.7	23.59103	0.0000
25		20	39.55	12.93903	
30		20	108.05	26.10198	

Figure 24 - Box plot showing mean number of eggs laid after multiple mating of freshly mated females of *N. rufipes* at 25 and 30 and 32.5^{0}C and photoperiod L:D 12:12

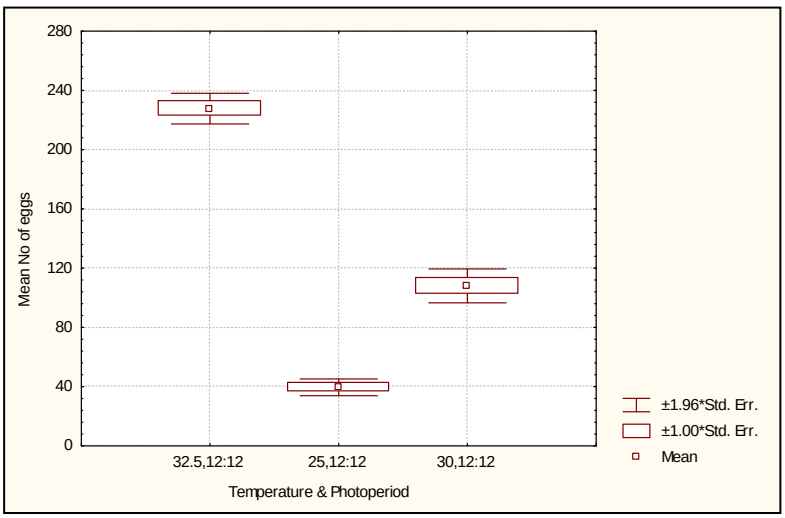

Figure 25 - Box plot showing mean number of eggs laid after double mating of freshly emerged *N. rufipes* at 32.5^{0}C and L:D 12:12

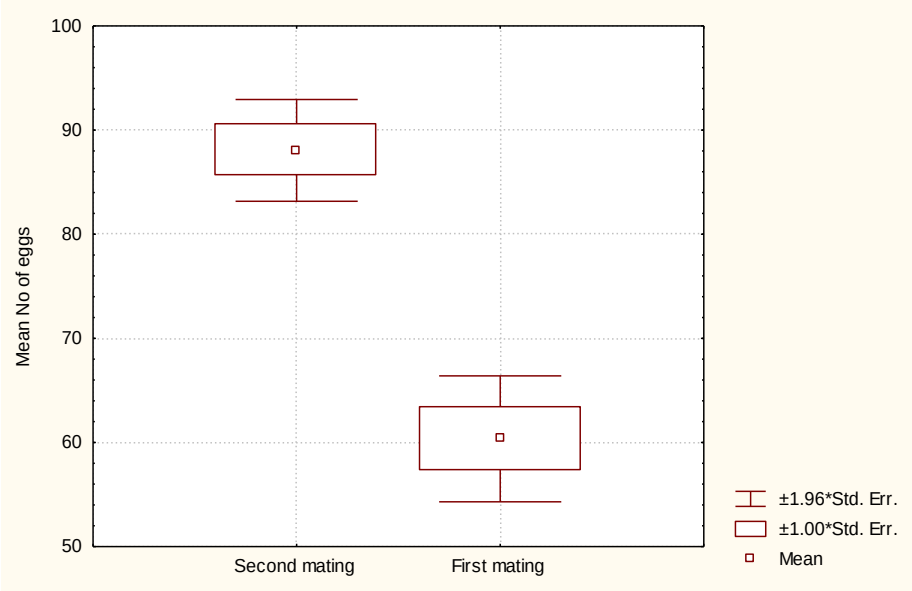

Figure 26 - Box plot showing mean number of eggs laid after double mating of 15-days old *N. rufipes* at 32.5^{0}C at L:D 12:12

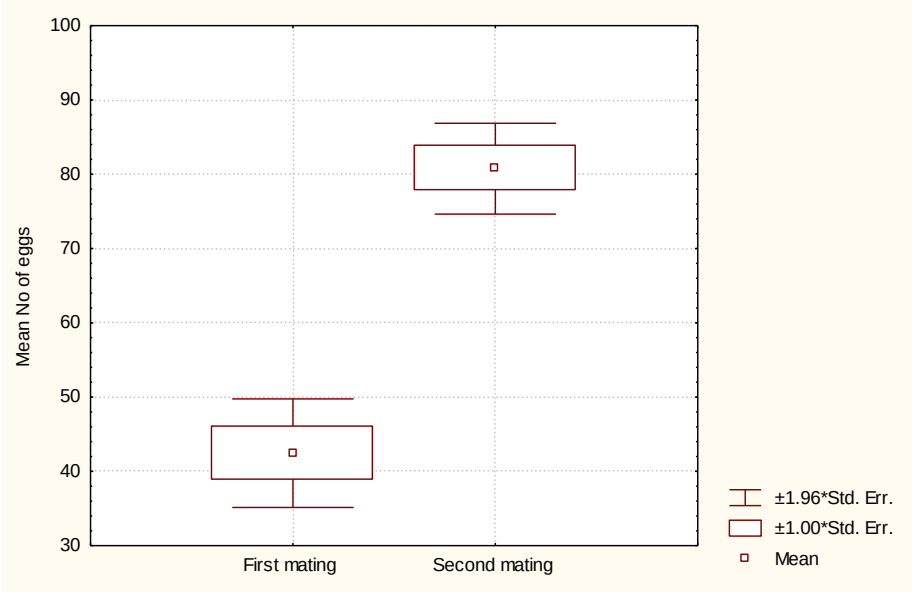

Table 79 - Mean number of eggs laid after multiple and double mating of 15-day old *N. rufipes* at 32.5^{0}C and L:D 12:12

	Valid N	Mean	Minimum	Maximum	Std.Dev.
Double mating	20	123.2	84	163	21.68094
Multiple mating	20	206.65	165	240	21.75044

Figure 27 - Box plot showing mean number of eggs laid after double and multiple mating of 15-days old *N.rufipes* at 32.5^{0}C and L:D 12:12

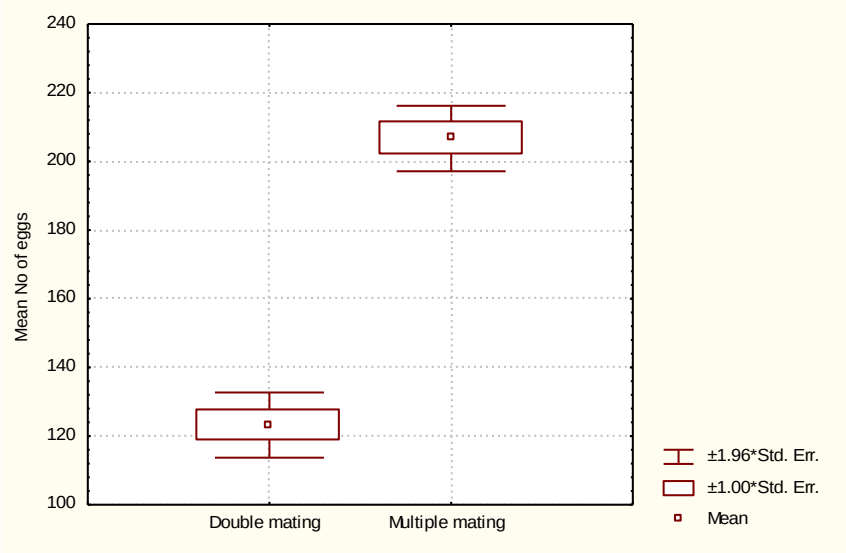

Table 80 - Mean number of eggs laid after multiple and double mating of freshly emerged *N.rufipes* at 32.5^{0}C and L:D 12:12

	Valid N	Mean	Minimum	Maximum	Std.Dev.
Double mating	20	148.4	116	189	20.76029
Multiple mating	20	227.7	179	264	23.59103

Figure 28 - Box plot showing mean number of eggs laid after double and multiple mating of freshly emerged *N.rufipes* at 32.5^{0}C and L:D 12:12

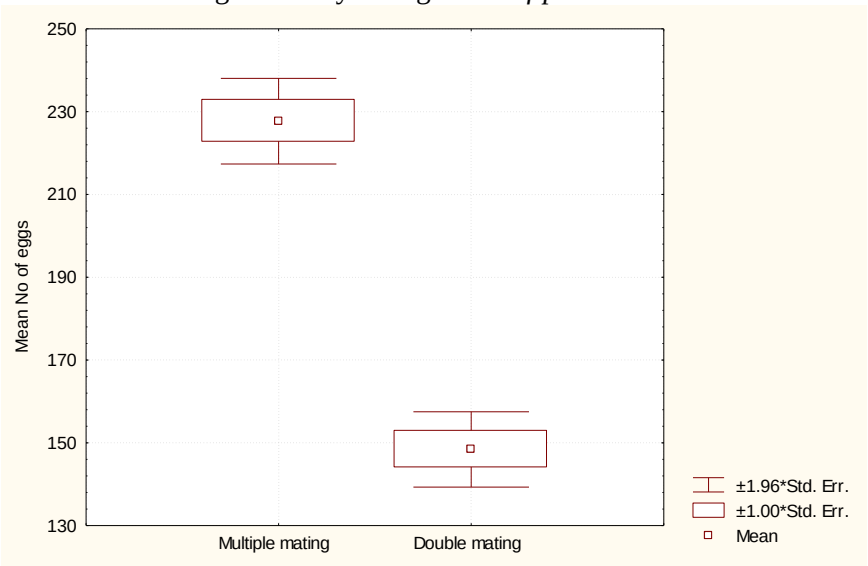

Figure 1- Box plot showing mean duration (in days) of developmental stages of *N. rufipes* at 32.5^{0}C and L:D 12:12

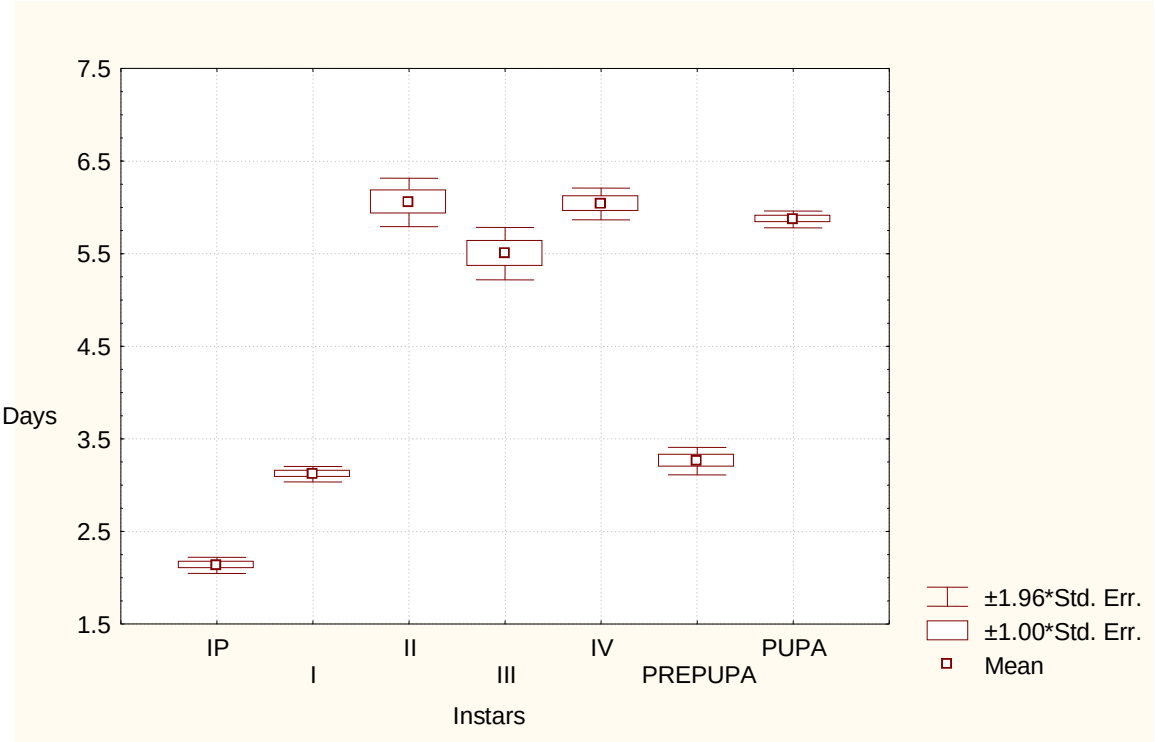

Table 81-Mean food consumption (in grams) of the larval instars of *N. rufipes* at 35^0C and L:D 12:12

Instar	Number	Mean	SD	p-level
First	17	0.0282	0.0095	7.56E-14
Second	17	0.0576	0.0120	
Third	16	0.0844	0.0203	
Total		**0.1702**		

Table 82 -ANOVA table giving significant difference between food consumption of different larval instars of *N. rufipes* at 35^0C, L:D 12:12

Temperature (^{0}C)	Photoperiod L:D	Instar			
			First	Second	Third
35	12:12	First		2.25E-06	7.83E-14
		Second	2.25E-06		1.8E-05
		Third	7.83E-14	1.8E-05	

Table 83-Mean food consumption (in grams) of the larval instars of *N. rufipes* at 32.5^0C and L:D 12:12

Instar	Number	Mean	SD	p-level
First	19	0.035789	0.010706	0.0000
Second	20	0.0715	0.016631	
Third	19	0.111053	0.015949	
Fourth	20	0.149	0.022919	
Total		**0.367342**		

Table 84 -ANOVA table giving significant difference between food consumption of different larval instars of *N. rufipes* at 32.5^0C and L:D 12:12

Temperature (^{0}C)	Photoperiod L:D	Instar				
			First	Second	Third	Fourth
32.5	12:12	First		2.53E-07	6.67E-20	1.67E-30
		Second	2.53E-07		1.44E-08	3.6E-21
		Third	6.67E-20	1.44E-08		4.82E-08
		fourth	1.67E-30	3.6E-21	4.82E-08	

Table 85-Details of total food consumption of the larval instars of *N. rufipes* at 30^0C and different photoperiods

Temperature (^{0}C)	Photoperiod L:D	Number	Mean	SD	p-level
30	24:0	70	0.217182	0.028321	**1.09E-08**
	0:24	72	0.371111	0.045571	
	12:12	58	0.301161	0.03448	

Table 86 -Mean food consumption (in grams) of the larval instars of *N. rufipes* at 30^0C and L:D 24:0

Instar	Number	Mean	SD	p-level
First	19	0.018947	0.005671	1.68E-28
Second	17	0.042941	0.011048	
Third	17	0.067647	0.012515	
Fourth	17	0.087647	0.012515	
Total		**0.217182**		

Table 87-ANOVA table giving significant difference between food consumption of different larval instars of *N.rufipes* at 30^0C and L: D 24:0

Temperature (^{0}C)	Photoperiod L:D	Instar				
			First	Second	Third	Fourth
30	24:0	First		1.43E-07	3.48E-19	5.11E-27
		Second	1.43E-07		1.35E-07	6.9E-17
		Third	3.48E-19	1.35E-07		1.75E-05
		fourth	5.11E-27	6.9E-17	1.75E-05	

Table 88 -Details of food consumption of the larval instars of *N. rufipes* at 30^0C and L:D 0:24

Instar	Number	Mean	SD	p-level
First	18	0.033889	0.007775	0.0000
Second	18	0.075	0.009235	
Third	18	0.114444	0.018856	
Fourth	18	0.147778	0.021572	
Total		**0.371111**		

Table 89 -ANOVA table giving significant difference between food consumption of different larval instars of *N. rufipes* at 30^0C and L: D 0:24

Temperature (^{0}C)	Photoperiod L:D	Instar				
			First	Second	Third	Fourth
30	0:24	First		9.89E-10	2.43E-22	0
		Second	9.89E-10		3.64E-09	4.59E-20
		Third	2.43E-22	3.64E-09		4.01E-07
		fourth	0	4.59E-20	4.01E-07	

Table 90 -Details of food consumption of the larval instars of *N. rufipes* at 30^0C and L:D 12:12

Instar	Number	Mean	SD	p-level
First	16	0.031875	0.006551	3.03E-28
Second	14	0.061429	0.007703	
Third	14	0.089286	0.012067	
Fourth	14	0.118571	0.014601	
Total		**0.301161**		

Table 91 -ANOVA table giving significant difference between food consumption of different larval instars of *N. rufipes* at 30^0C and L: D 12:12

Temperature (^{0}C)	Photoperiod L:D	Instar				
			First	Second	Third	Fourth
30	12:12	First		1.23E-08	5.54E-19	3.05E-27
		Second	1.23E-08		1.36E-07	2.69E-18
		Third	5.54E-19	1.36E-07		3.76E-08
		fourth	3.05E-27	2.69E-18	3.76E-08	

Figure 29 - Box plot showing mean number of eggs laid after first and second mating in double mating of freshly mated at 25^0C L:D 12:12

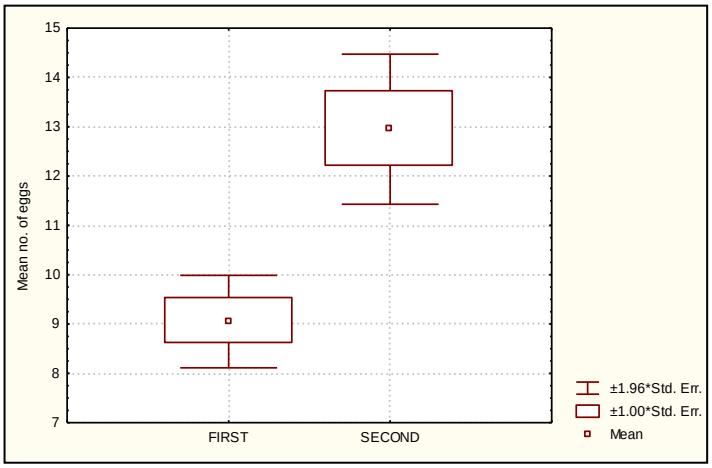

Figure 30 - Box plot showing mean number of eggs laid after double and multiple of freshly emerged females at 25^0C L:D 12:12

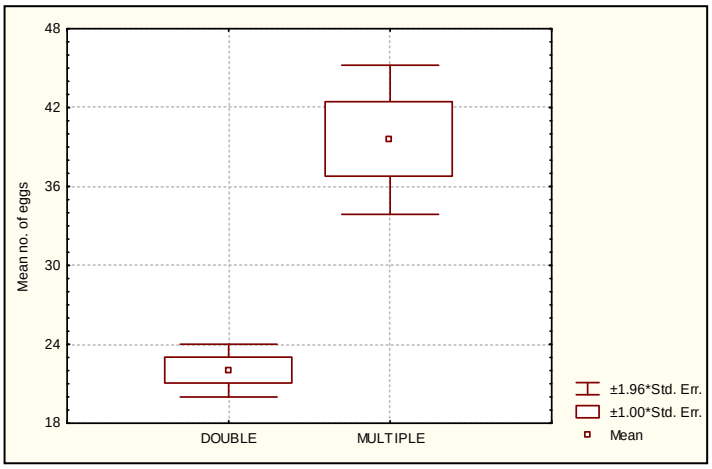

Figure 31 - Box plot showing mean number of eggs laid after first and second mating in double mating of freshly mated females at 25^0C L:D 0:24

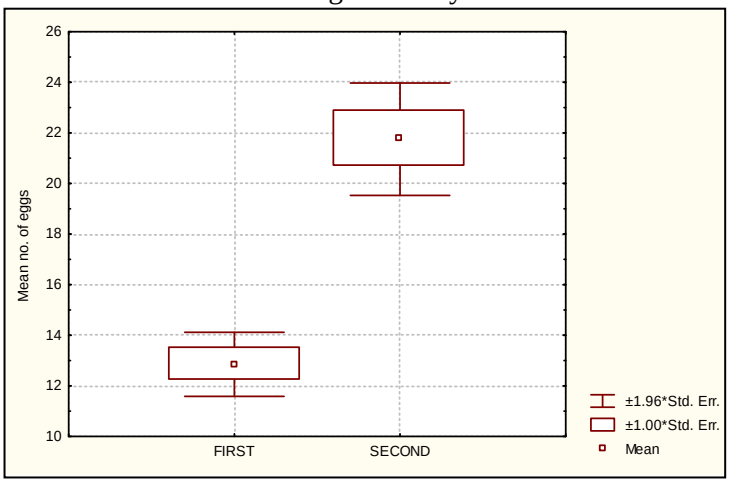

Figure 32 - Box plot showing mean number of eggs laid after double and multiple mating of freshly mated females at 25^0C L:D 0:24

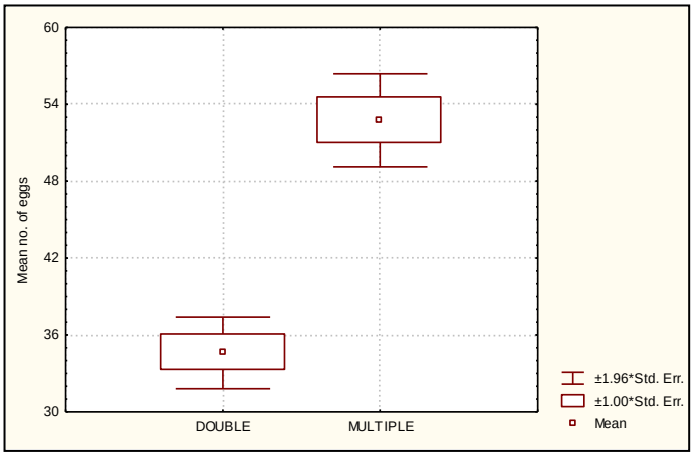

Figure 33 - Box plot showing mean number of eggs laid after first and second mating in double mating of freshly emerged females at 25^0C L:D 24:0

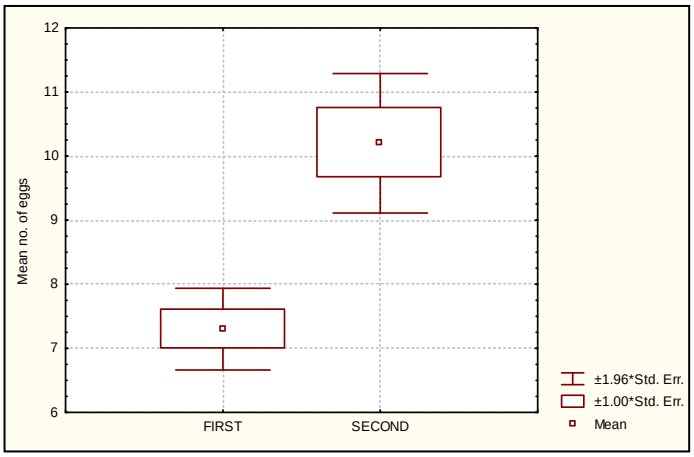

Figure 34 - Box plot showing mean number of eggs laid after double and multiple mating of freshly mated females at 25^0C L:D 24:0

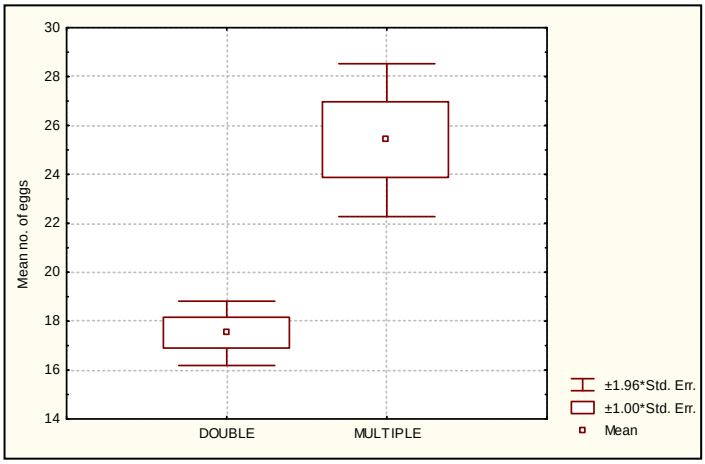

Figure 35-Egg laying peak of freshly mated *N. rufipes* at 25^0C and L:D 24:0 after multiple mating

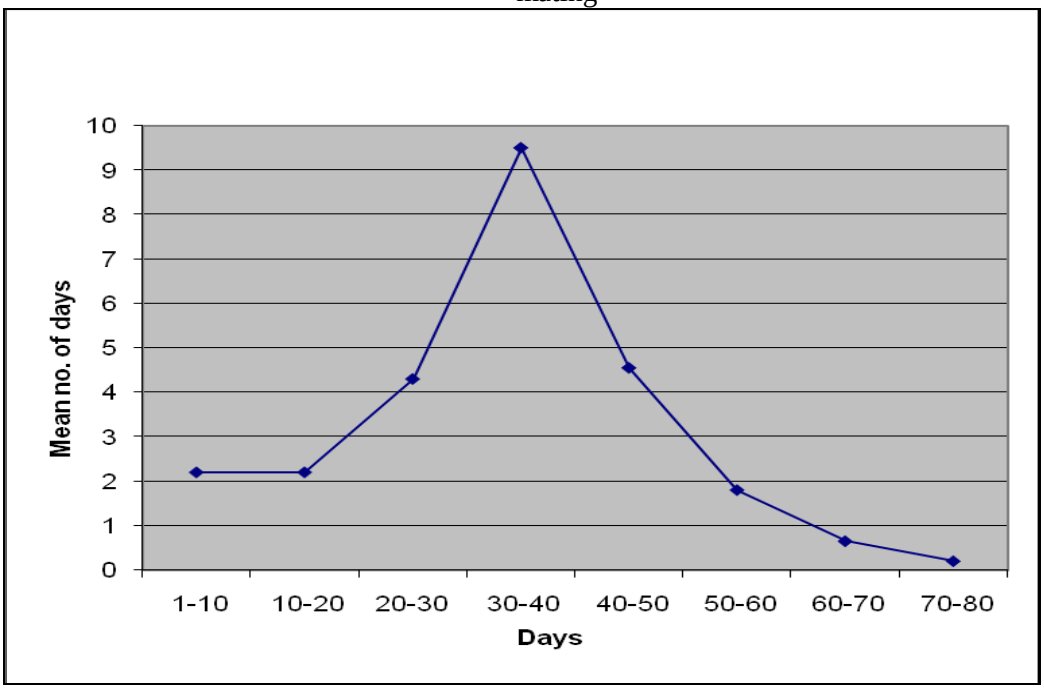

Figure 36 -Egg laying peak of freshly mated *N. rufipes* at 25^0C and L:D 0:24 after multiple mating

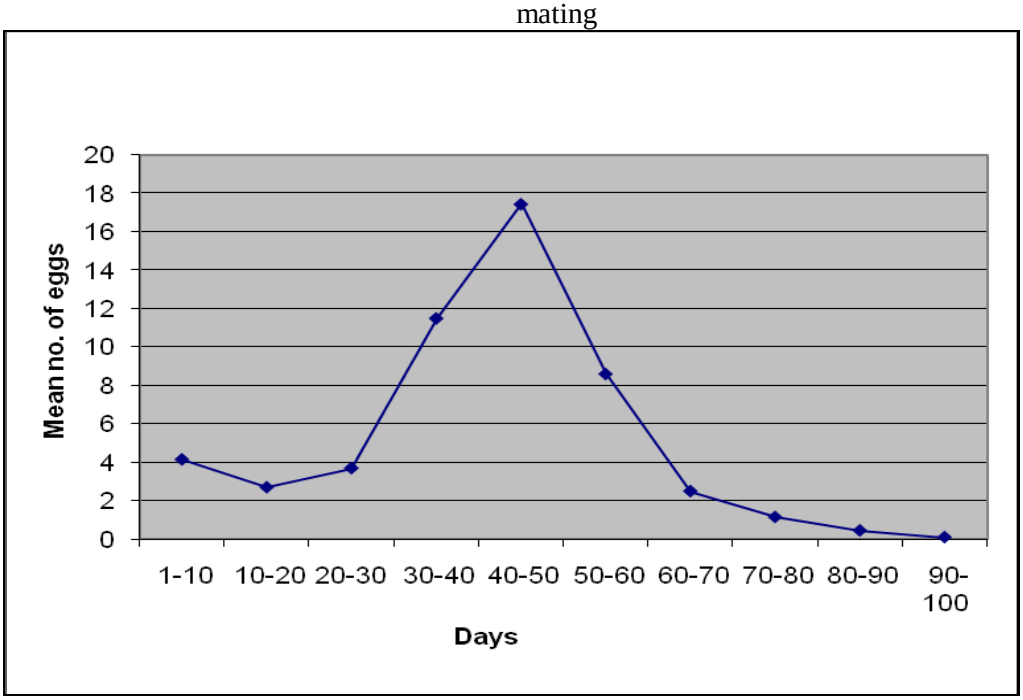

Figure 37 -Egg laying peak of freshly mated *N. rufipes* at 25^{0}C and L:D 12:12 after multiple mating

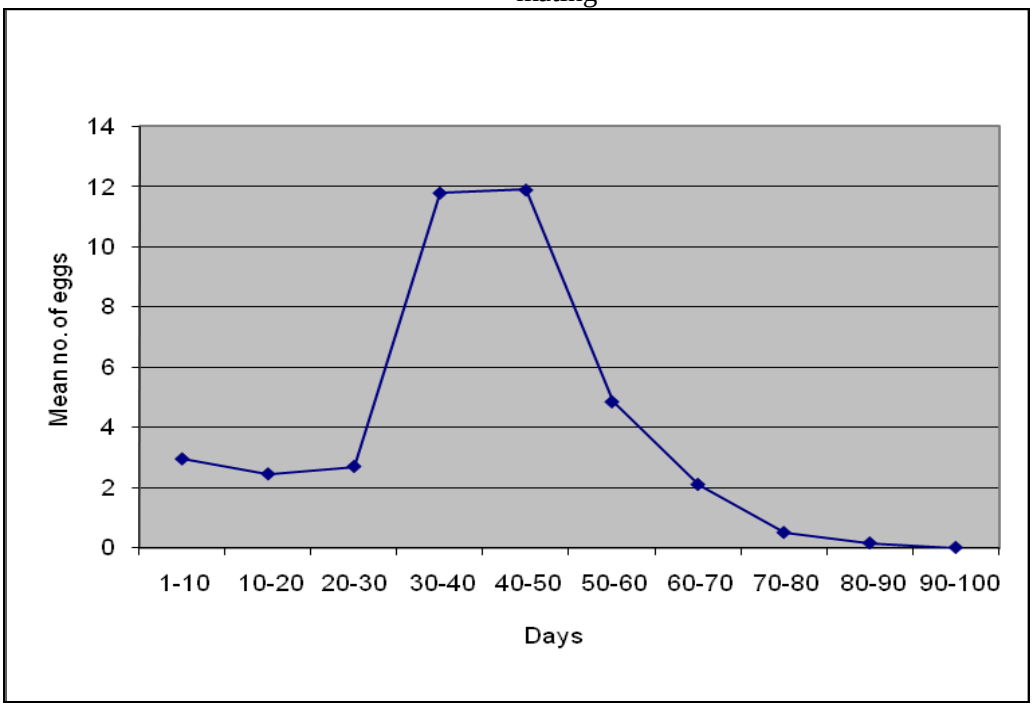

Figure 38 - Box plot showing mean number of eggs laid during first and second mating after double mating at 30^{0}C L:D 12:12

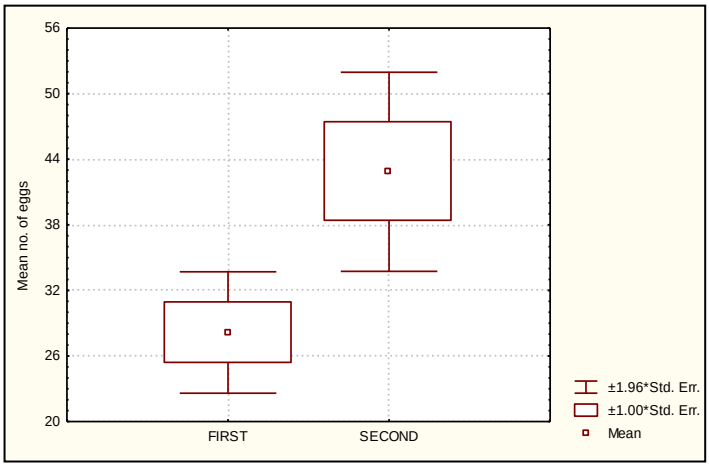

Figure 39 - Box plot showing mean number of eggs laid after double and multiple at 30^0C L:D 12:12

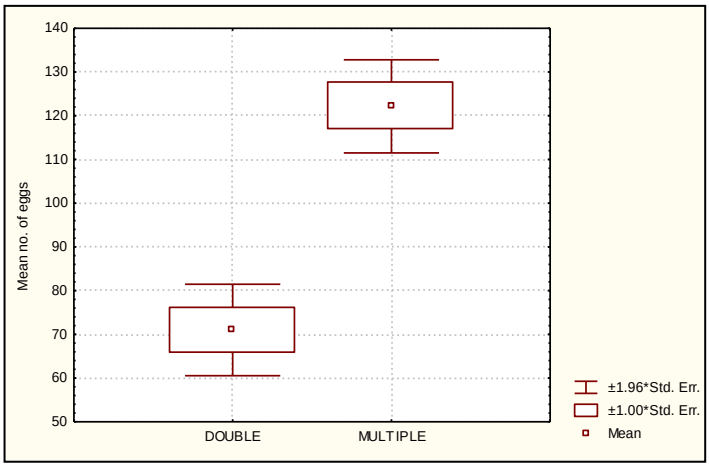

Figure 40 - Box plot showing mean number of eggs laid after first and second mating in double mating at 30^0C L:D 0:24

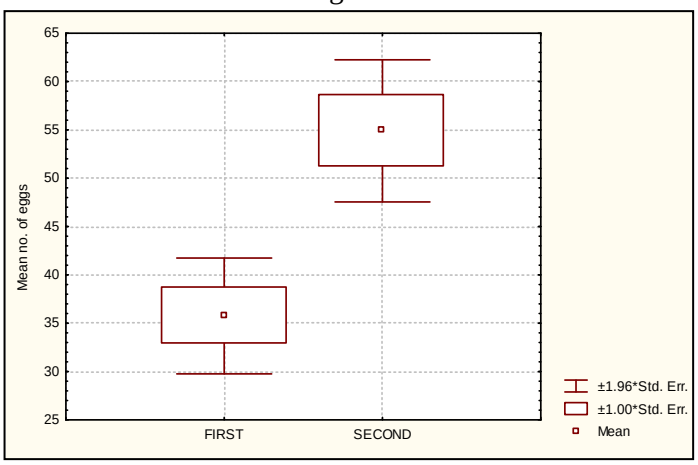

Figure 41 - Box plot showing mean number of eggs laid after double and multiple at 30^0C L: D 0:24

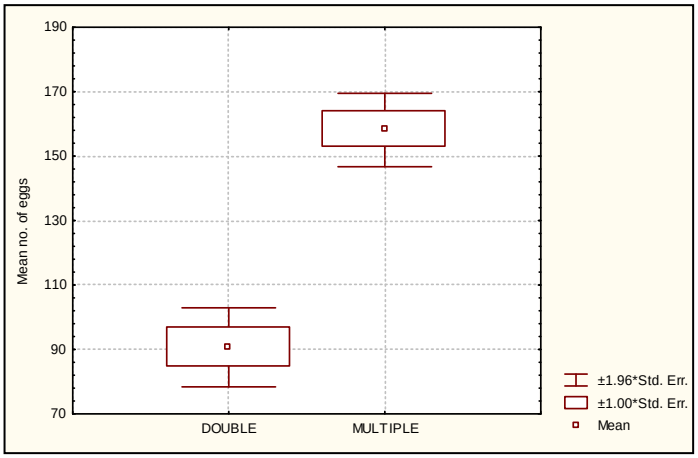

Figure 42 - Box plot showing mean number of eggs laid after first and second mating in double mating at 30^0C L:D 24:0

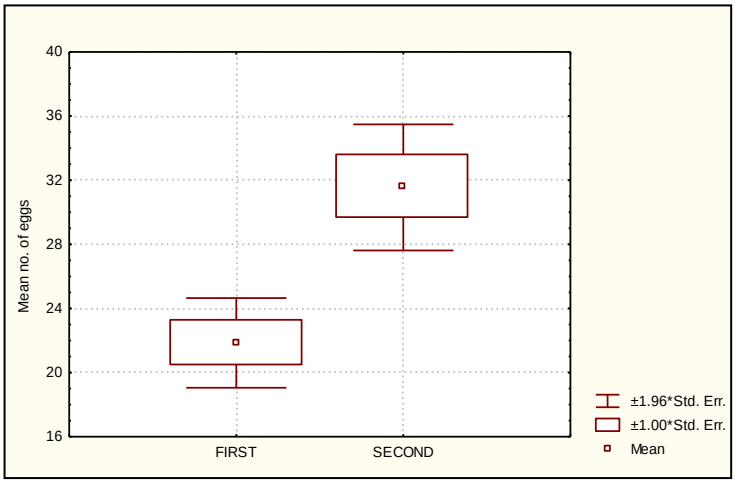

Figure 43 - Box plot showing mean number of eggs laid after double and multiple at 30^0C L:D 24:0

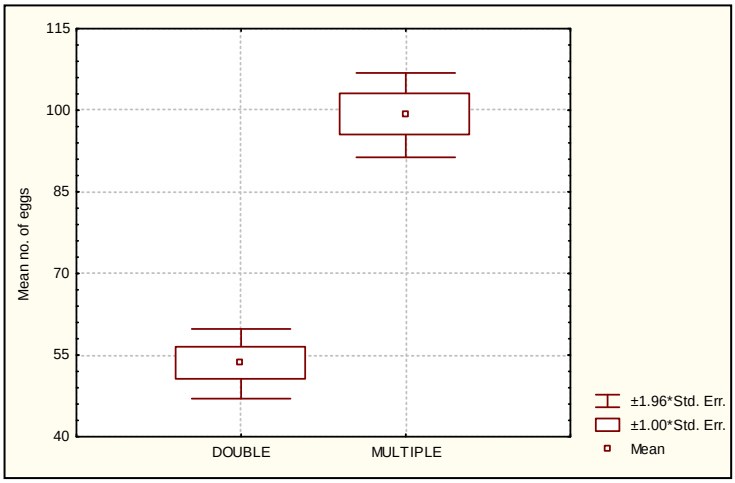

Figure 44 -Egg laying peak of freshly mated *N. rufipes* at 30^0C and L:D 12:12 after multiple mating

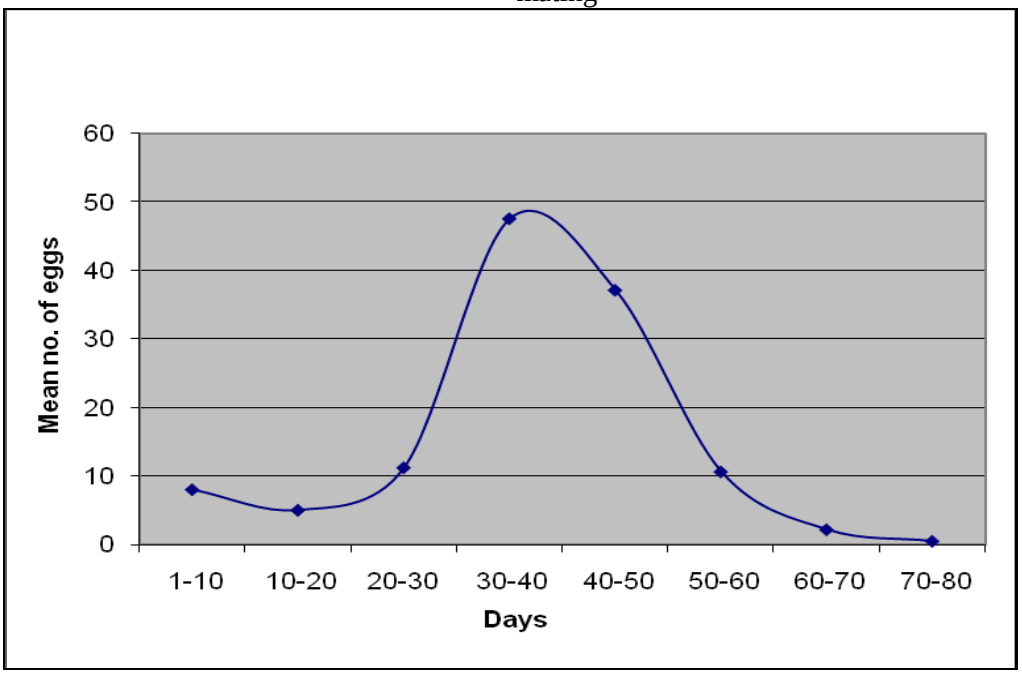

Figure 45 -Egg laying peak of freshly mated *N. rufipes* at 30^0C and L:D 0:24 after multiple mating

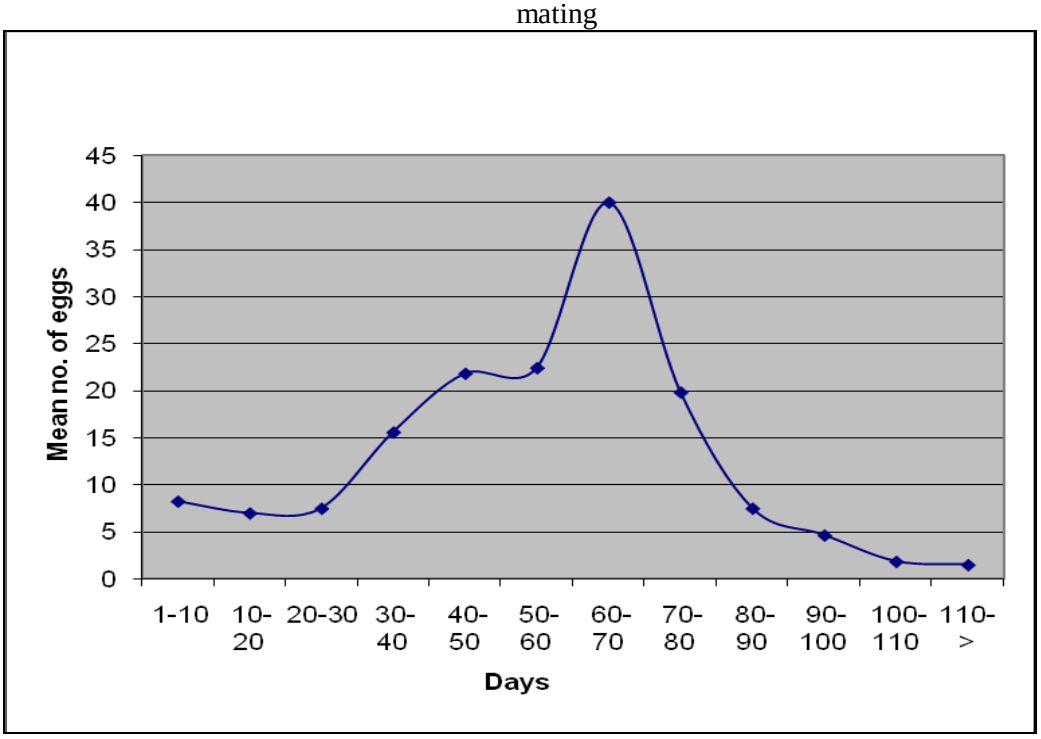

Figure 46 -Egg laying peak of freshly mated *N. rufipes* at 30^0C and L:D 24:0 after multiple mating

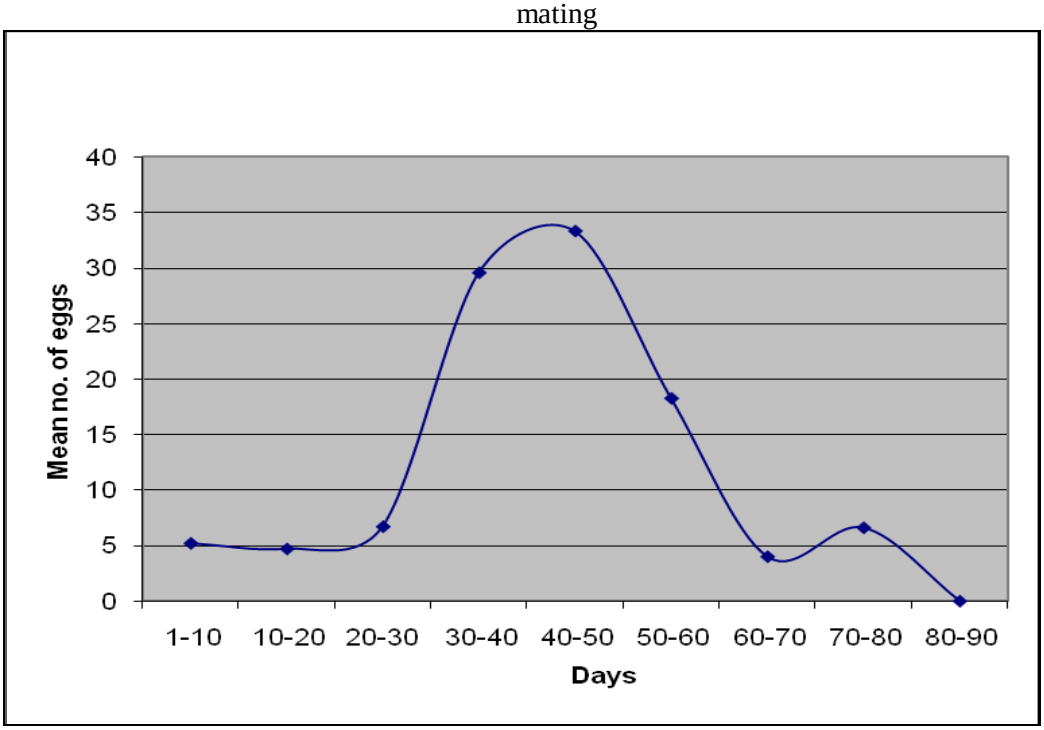

Figure 47 -Egg laying peak of freshly mated *N. rufipes* at 32.5^0C and L:D 12:12 after multiple mating

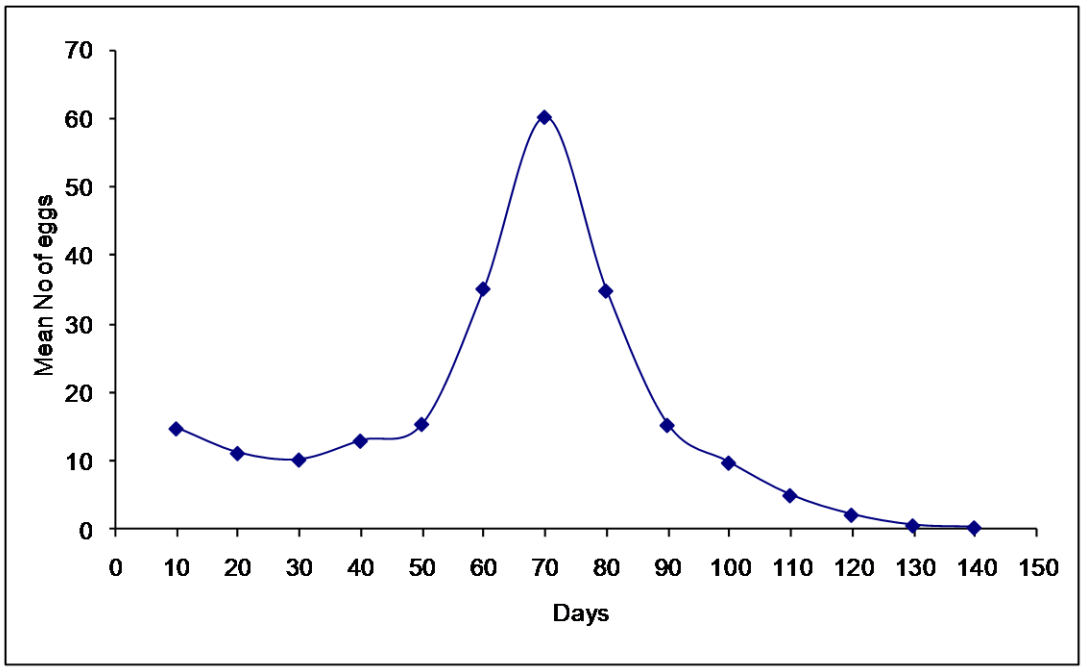

Figure 48 -Egg laying peak of 15-day old mated *N. rufipes* at 32.5^{0}C and L:D 12:12 after multiple mating

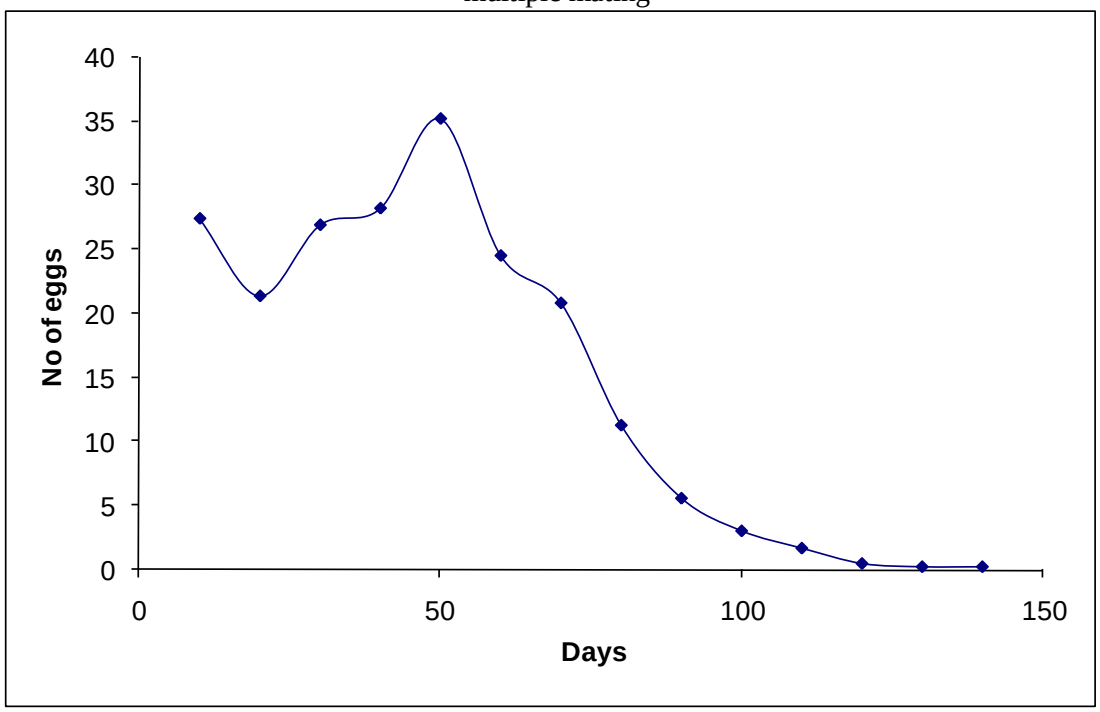

Fig 49 - Probit of kill of 3rd instar of *N. rufipes* and concentration of black pepper oil showing probit regression line

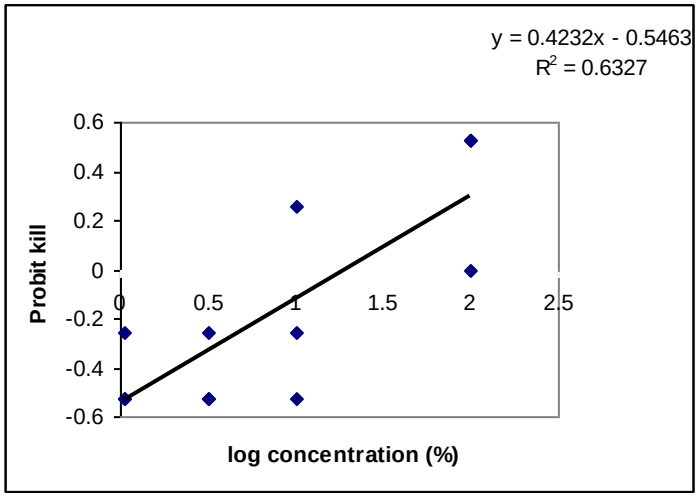

Fig 50 - Probit of kill of 4th instar of *N. rufipes* and concentration of black pepper oil showing probit regression line

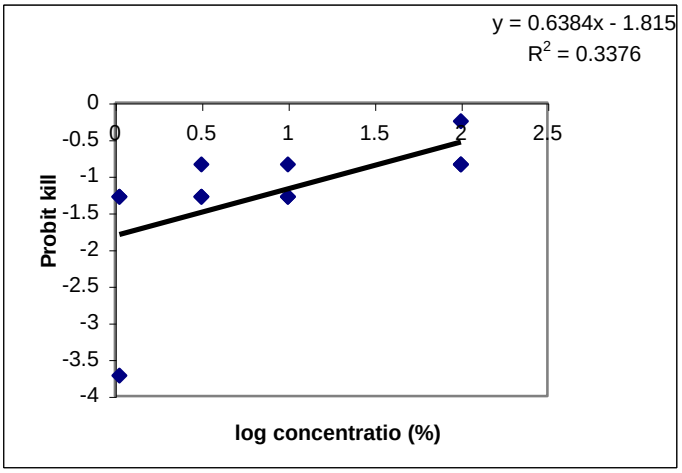

Fig 51 - Probit of kill of adult of *N. rufipes* and concentration of black pepper oil showing probit regression line

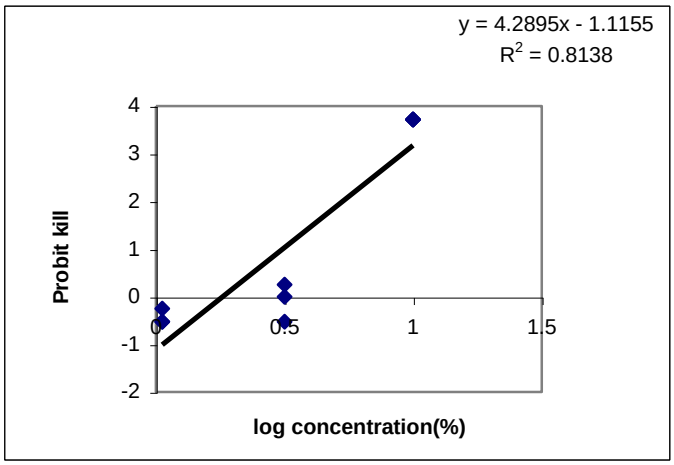

Fig 52 - Probit of kill of 3rd instar of *N. rufipes* and concentration of clove oil showing probit regression line

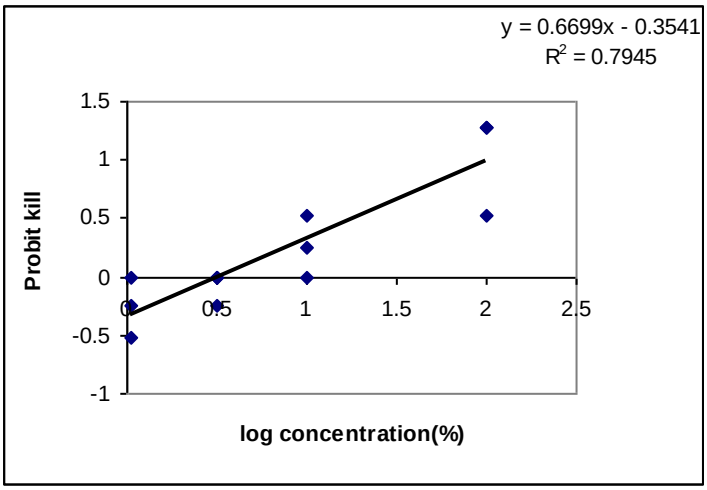

Fig 53 - Probit of kill of 4th instar of *N. rufipes* and concentration of clove oil showing probit regression line

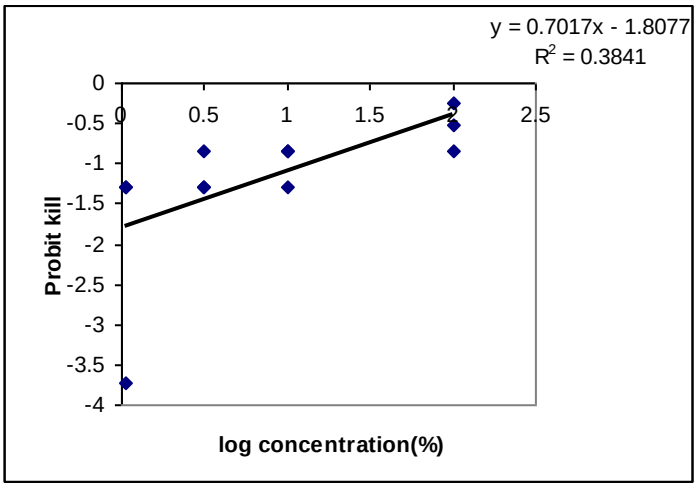

Fig 54 - Probit of kill of adult of *N. rufipes* and concentration of clove oil showing probit regression line

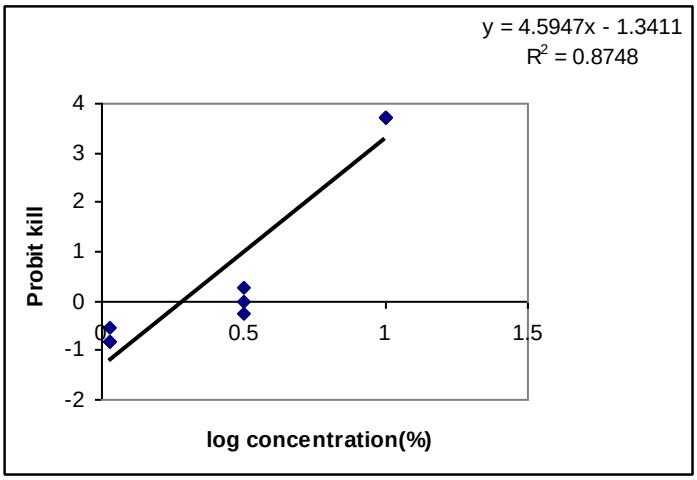

Fig 55 - Probit of kill of 3rd instar of *N. rufipes* and concentration of crude lemon grass oil showing probit regression line

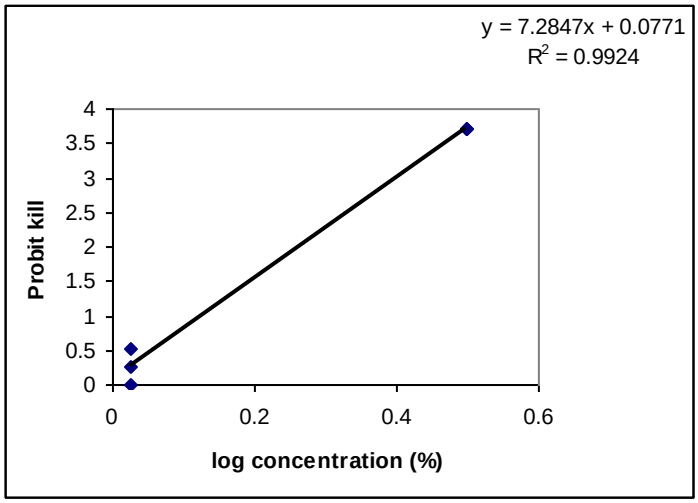

Fig 56 - Probit of kill of 4th instar of *N. rufipes* and concentration of crude lemon grass oil showing probit regression line

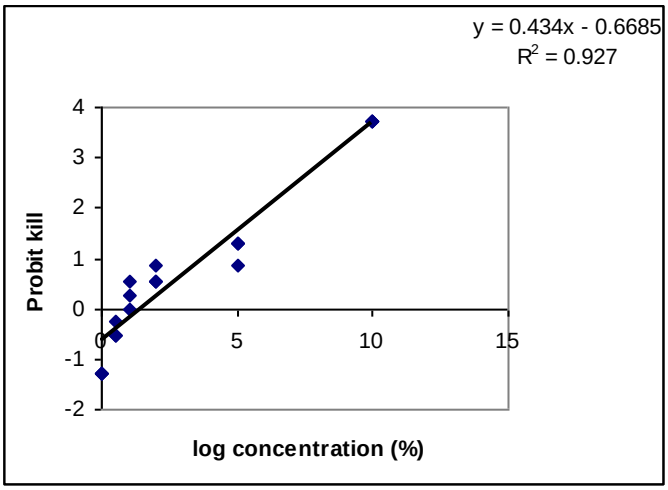

Fig 57 - Probit of kill of adult of *N. rufipes* and concentration of crude lemon grass oil showing probit regression line

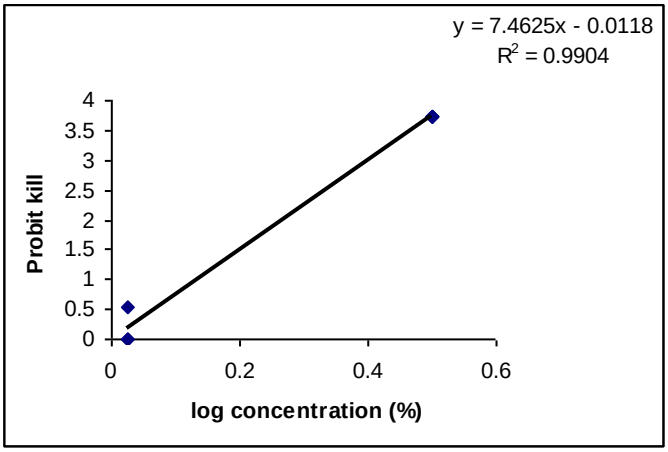

Fig 58 - Probit of kill of 3rd instar of *N. rufipes* and concentration of lemon grass oil-A showing probit regression line

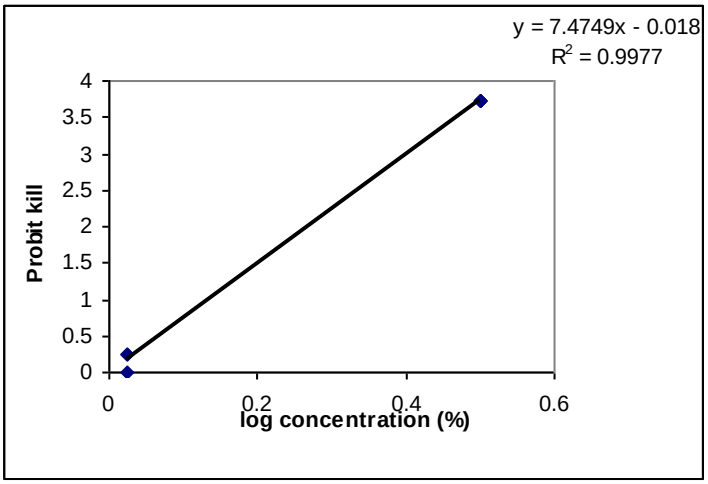

Fig 59 - Probit of kill of 4th instar of *N. rufipes* and concentration of lemon grass oil-A showing probit regression line

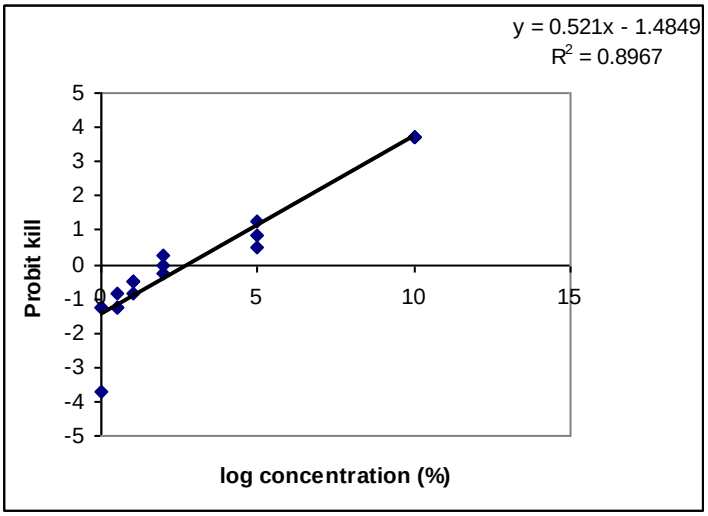

Fig 60 - Probit of kill of adult of *N. rufipes* and concentration of lemon grass oil-A showing probit regression line

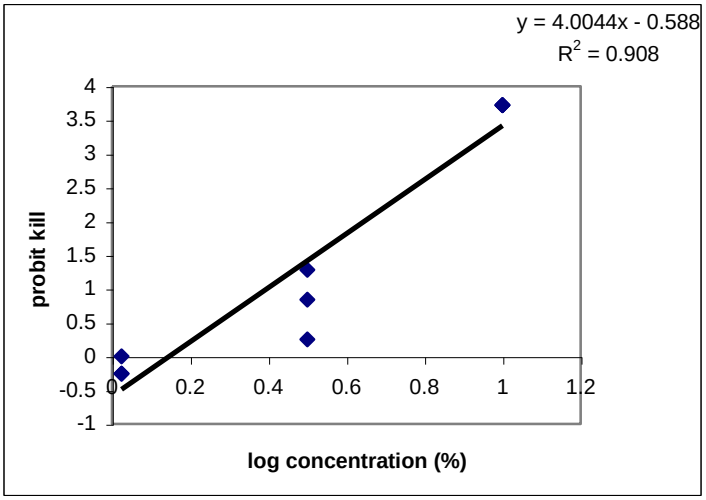

Fig 61- Probit of kill of 3rd instar of *N. rufipes* and concentration of lemon grass oil-B showing probit regression line

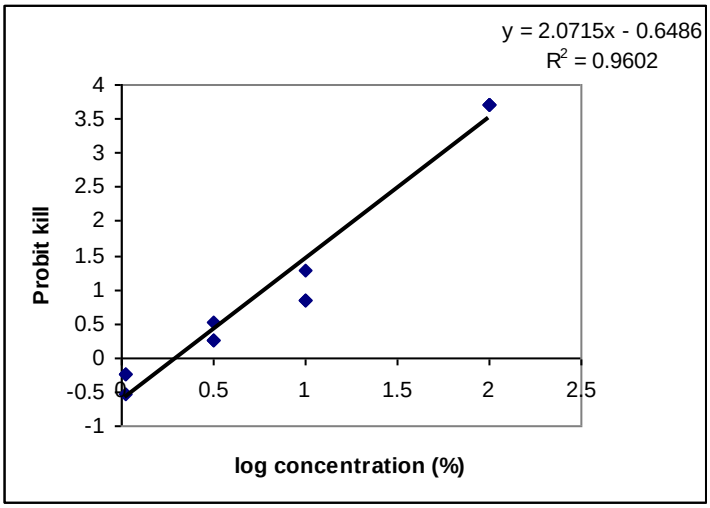

Fig 62 - Probit of kill of 4th instar of *N. rufipes* and concentration of lemon grass oil-B showing probit regression line

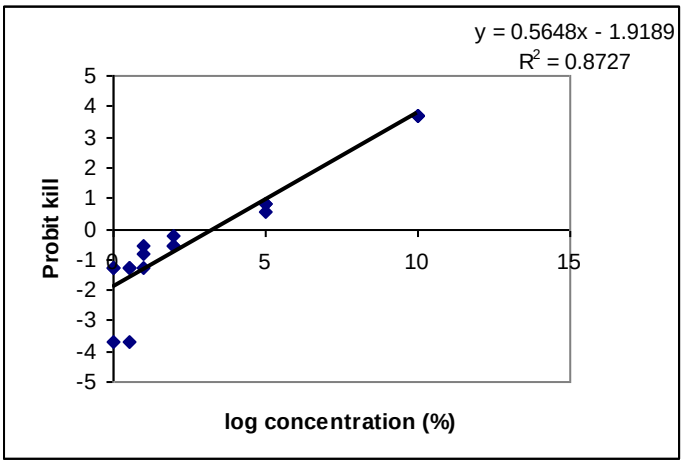

Fig 63 - Probit of kill of adult of *N. rufipes* and concentration of lemon grass oil-B showing probit regression line

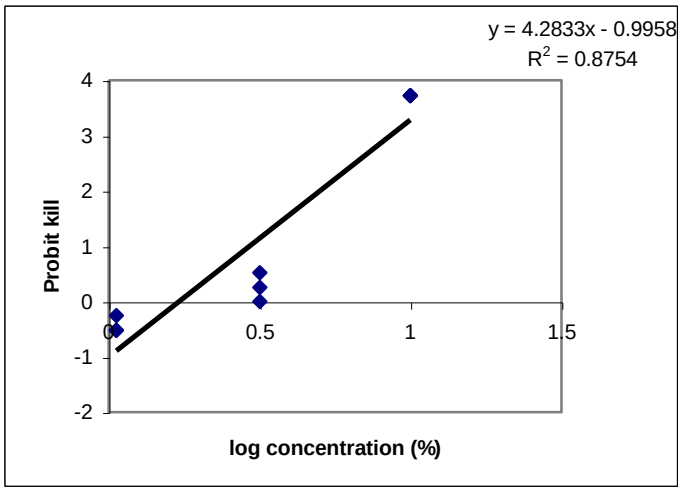

Fig 64- Probit of kill of 3rd instar of *N. rufipes* and concentration of turmeric showing probit regression line

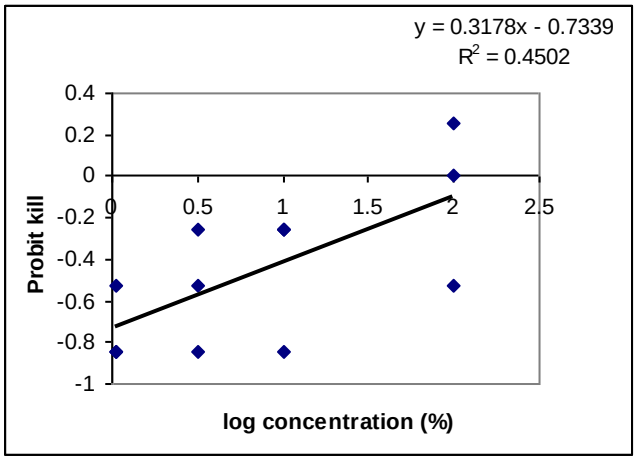

Fig 65 - Probit of kill of 4th instar of *N. rufipes* and concentration of turmeric showing probit regression line

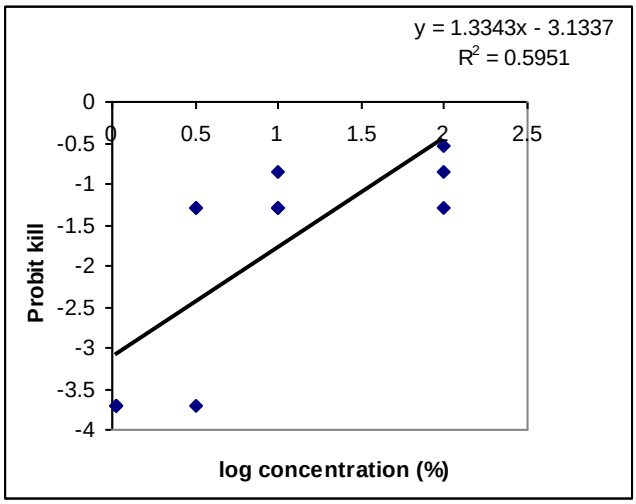

Fig 66 - Probit of kill of adult of *N. rufipes* and concentration of turmeric showing probit regression line

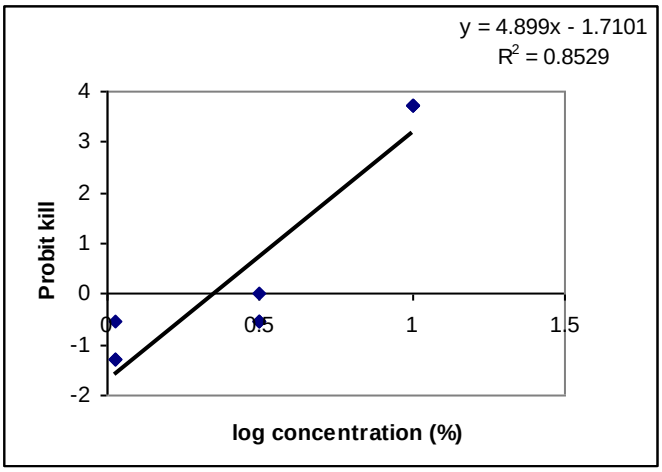

Fig 67- Probit of kill of 3rd instar of *N. rufipes* and concentration of cinnamon showing probit regression line

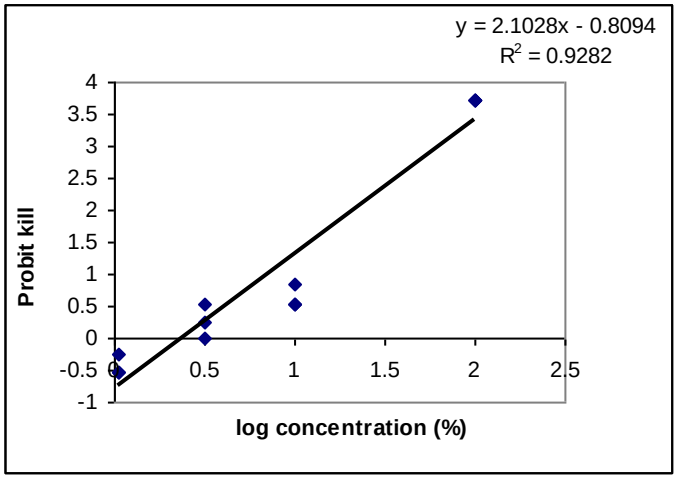

Fig 68 - Probit of kill of 4th instar of *N. rufipes* and concentration of cinnamon showing probit regression line

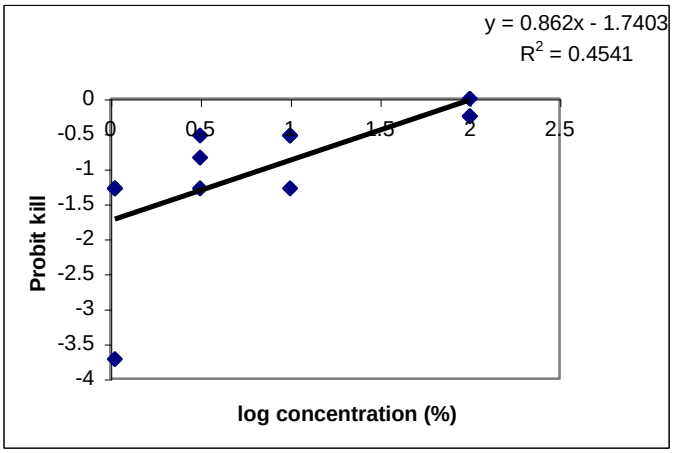

Fig 69 - Probit of kill of adult of *N. rufipes* and concentration of cinnamon showing probit regression line

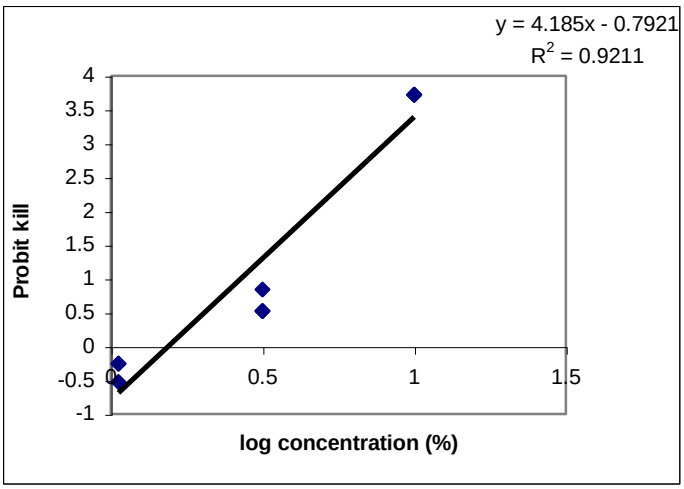

Figure 70- Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of black pepper oil

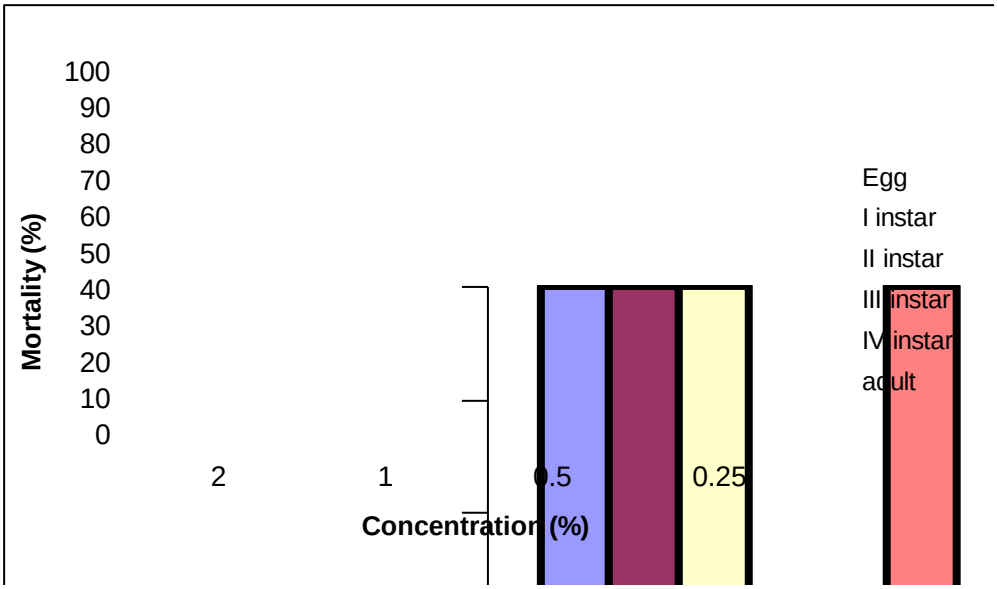

Figure 71 -Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of clove oil

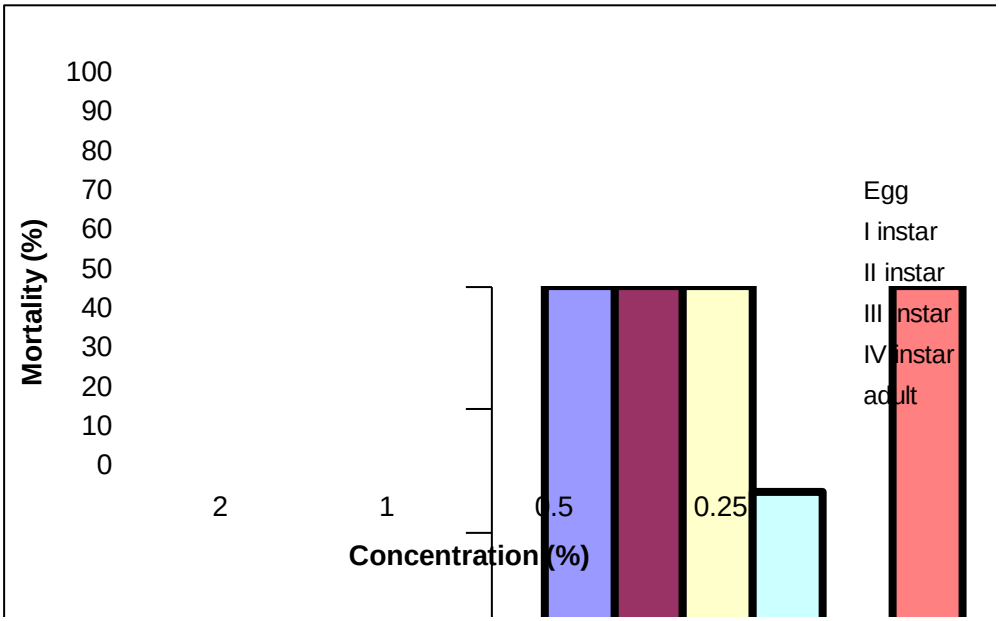

Figure 72 -Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of Crude Lemon Grass Oil (CLGO)

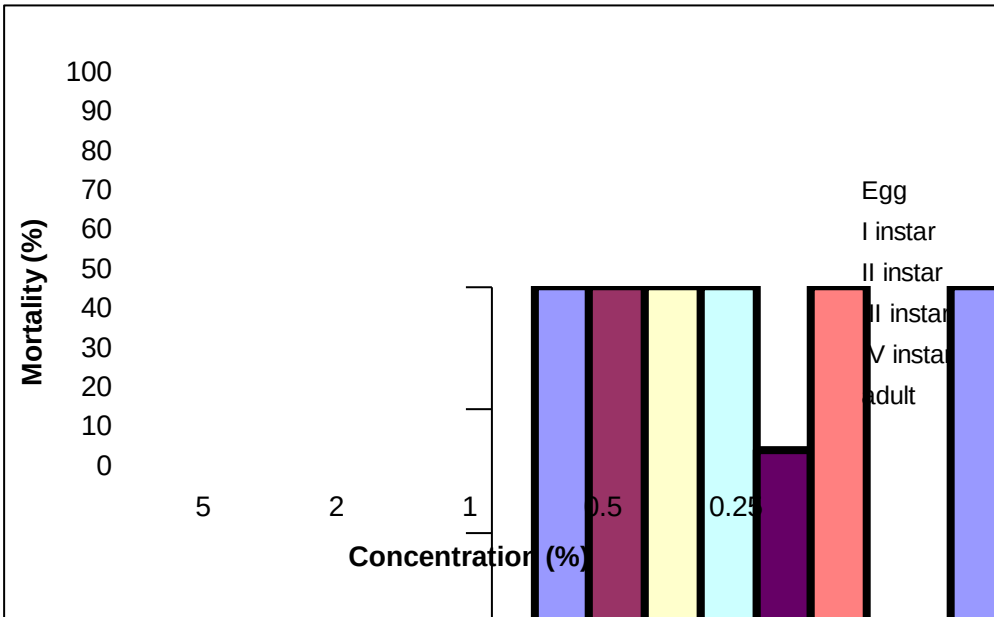

Figure 73-Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of Lemon Grass Oil-A (LGO-A)

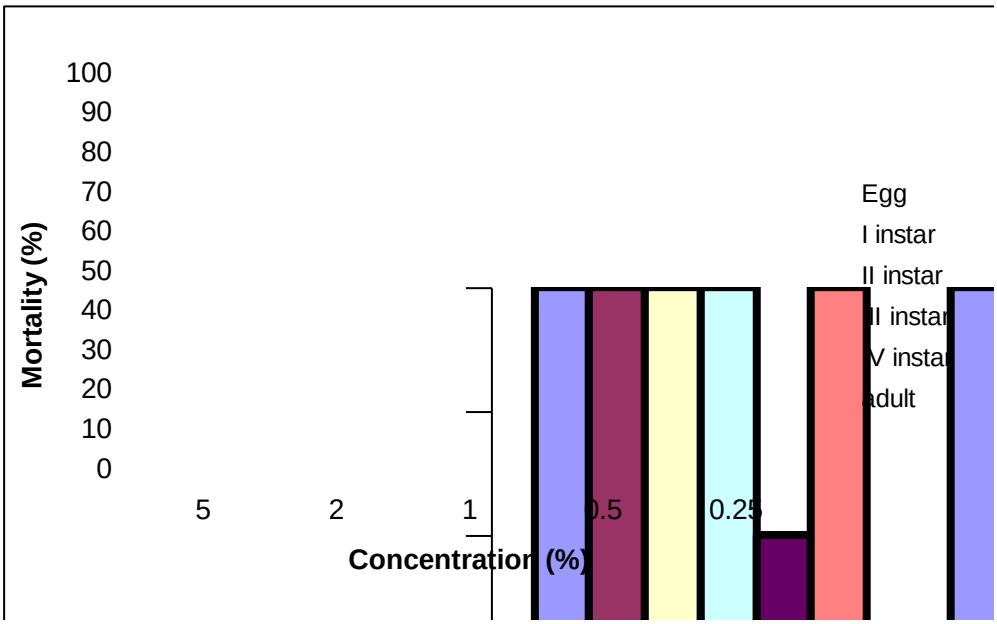

Figure 74-Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of Lemon Grass Oil-B (LGO-B)

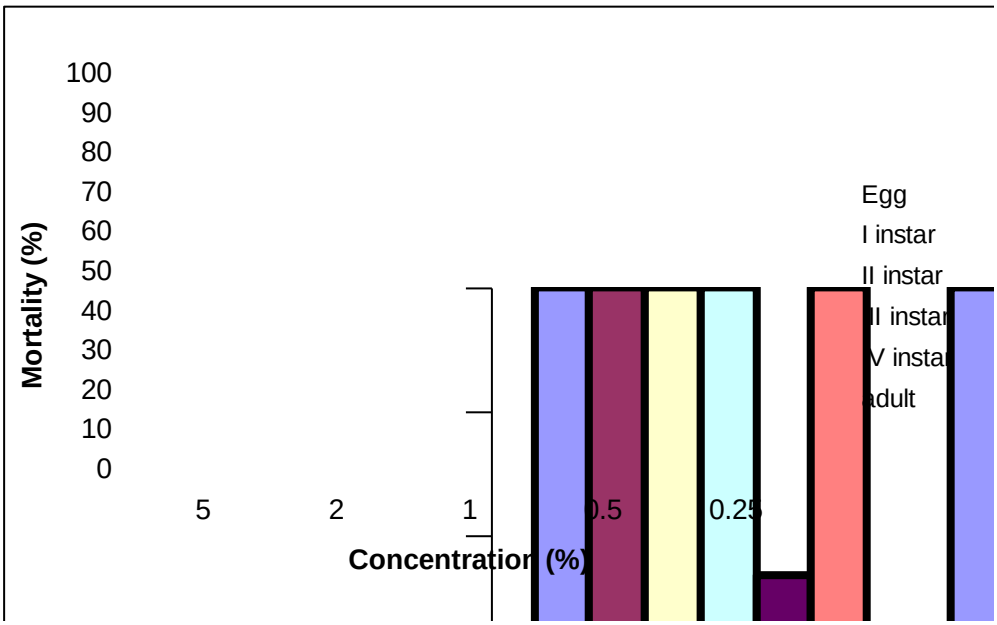

Figure 75 -Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of Turmeric oil

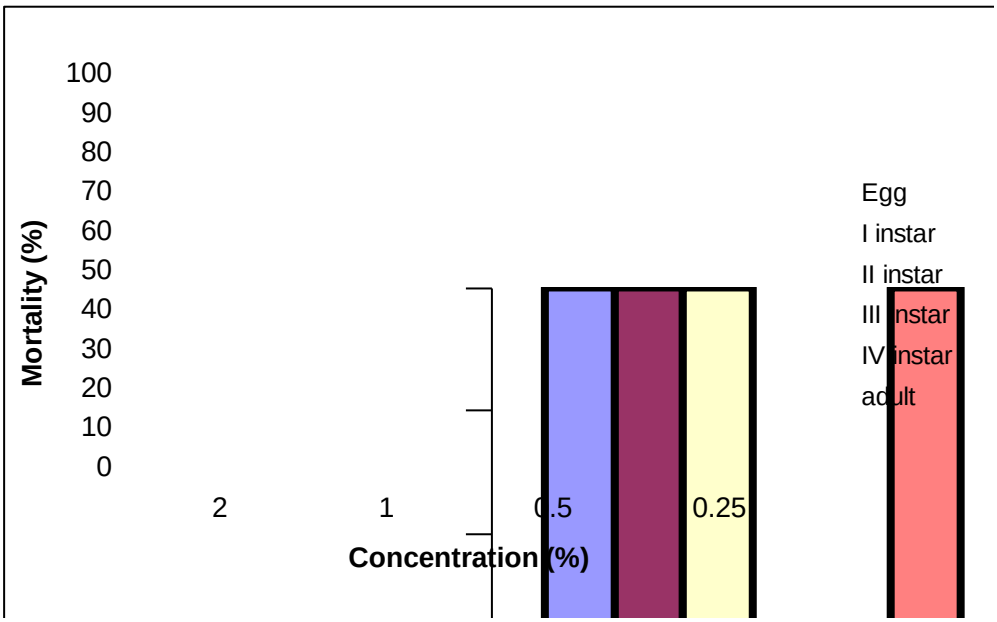

Figure 76 -Percentage mortality of the developmental stages of *N. rufipes* at different concentrations of Cinnamon oil

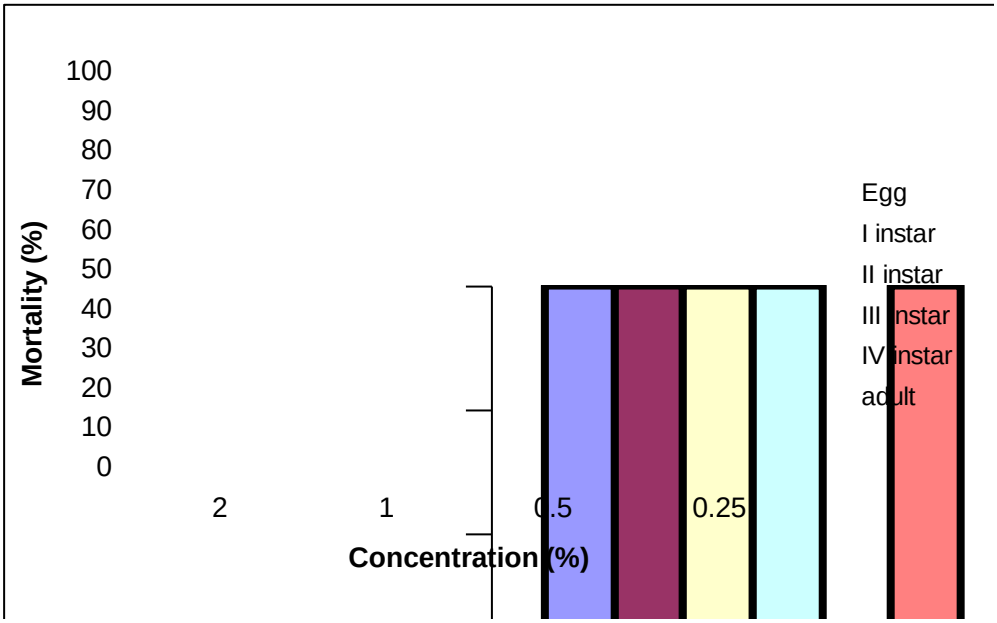

Table – 92. Significant difference between effects of different oils

Oil	Black Pepper	Clove	Crude lemon grass	Lemon grass-A	Lemon grass-B	Turmeric	Cinnamon
Black Pepper		0.999822	**0.008202**	0.068024	0.319563	0.997953	0.952495
Clove	0.999822		**0.036312**	0.197962	0.592915	0.969378	0.995453
Crude lemon Grass	**0.008202**	**0.036312**		0.994528	0.816754	**0.000501**	0.252376
Lemon grass-A	0.068024	0.197962	0.994528		0.992761	**0.007694**	0.647957
Lemon grass –B	0.319563	0.592915	0.816754	0.992761		0.071317	0.951984
Turmeric	0.997953	0.969378	**0.000501**	**0.007694**	0.071317		0.696234
Cinnamon	0.952495	0.995453	0.252376	0.647957	0.951984	0.696234	