Pollination biology of selected taxa of the tribe Commelineae (Commelinaceae)

Thesis submitted to
the University of Calicut in partial fulfilment of
the requirement for the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

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SEPTEMBER 2020

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Certificate

This is to certify that the thesis entitled, "**Pollination biology of selected taxa of the tribe Commelineae (Commelinaceae)**" submitted to the University of Calicut, for the award of the degree of **Doctor of Philosophy** in **Botany**, is a bona fide record of the original research work carried out by Ms. Veena V., at Angiosperm Taxonomy and Floristics Division, Department of Botany, University of Calicut, under my supervision and guidance. No part of the present work has formed the basis for the award of any other degree/diploma to any candidate of any University previously.

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25.09.2020

Prof. Santhosh Nampy
Supervising Teacher

Declaration

I, Veena V., hereby declare that the thesis entitled **"Pollination biology of selected taxa of the tribe Commelineae (Commelinaceae)"** submitted to the **University of Calicut**, for the award of the degree of **Doctor of Philosophy** in **Botany** is a bona fide record of the original research work carried out by me under the supervision and guidance of Dr. Santhosh Nampy, Professor, Department of Botany, University of Calicut, and that it has not been submitted earlier, either in part or full for the award of any degree/diploma to any candidate of any University.

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25.09.2020

Acknowledgements

It is with immense gratitude that I acknowledge my supervisor Dr. Santhosh Nampy, Professor, Department of Botany, University of Calicut, for his invaluable guidance, boundless patience, continuous motivation, encouragement, inspiration and support throughout my research period. Without the guidance and persistent help he offered, I would have been unable to complete the work.

I express my sincere gratitude to Prof. V.V. Radhakrishnan, Head, Department of Botany and to Prof. John E. Thoppil and Prof. K.M. Jayaram, former Heads, Department of Botany, University of Calicut, for providing necessary facilities to carry out this work.

I take this opportunity to express my gratitude and appreciation to Dr. K.V. Mohanan, former Head, Department of Botany, University of Calicut, for guidance and help in carrying out the statistical analysis needed for my work.

I gratefully acknowledge the Kerala State Council for Science Technology and Environment, for awarding a research fellowship, enabling me to carry out my work smoothly.

Ii is my pleasure to thank Dr. C.J. Mani, Dr. Abis V. Cherusseri, Dr. Sibichan M. Thomas, Dr. Sevichan P.J., Dr. Jojo joseph, Dr. George P.S., and the Late Dr. Jose Mary Das (St. Joseph's College Devagiri) for their support, kindness and encouragement as well as the inspiration.

I extend my thanks to the staff and teachers of the Department of Botany, University of Calicut, for their support, feedback and also for providing the necessary facilities.

I am grateful to Dr. Santhosh Srivihar, member trustee of Trust for Animal Taxonomy, Calicut (C/o Zoological Survey of India), Shameem K.M., Shyamna K. Baboo and Rajesh K.M, Department of Zoology, University of Calicut, for the help rendered during the identification of insect specimens.

I extend my thanks to Dr. P.M. Prakasan (Former Librarian) and Mrs. Soni Krishnan, Librarian, Department of Botany, University of Calicut, for availing library facilities.

I have great pleasure in acknowledging the invaluable help and constant encouragements extended by my lab mates, Dr. Manudev K.M., Dr. Janeesha A.P., Mr. Arunkumar P.G., Mr. Syam Radh S., Ms. Resmi S., Mrs. Dani Francis, Ms., Divya K. Venugopal, Mr. Vishnu Mohan, Mrs. Sreekutty T. K., Ms. Krishnapriya M.P., Mr. Akhil M.K., Ms. Aneesha, Ms. Dilna P.K., Ms. Priyanka T., Mr. Anvar K., Mr. Rajeevan and Mr. Saleem C. Thank you for making each day effortless, friendly and enjoyable.

I gratefully acknowledge Ms. Smisha K.P. and Dr. Aswani K., senior research fellows, Department of Botany, University of Calicut for their help and guidance, especially during the initial phases of my work. I am also thankful to Dr. Ajayan, Research associate, Department of Botany, University of Calicut for his help and valuable suggestions.

I am also grateful to Ms. Drisya V., Ms. Geethika K., Ms. Jeomol, Mr. Nikhil K., Ms. Amrutha A., Mr. Jayakrishnan T., Mr. Shinoj K.and Mr. Rajeesh E.P. (Research scholars, Angiosperm taxonomy division, Department of Botany, University of Calicut)

A special mention goes to Ms. Swetha K., Mr. Arun T. Ram, Ms. Nimly and Mr. Nidheesh Roy, for their friendly support.

I would like to thank Mr. Midhun M.K. (Unni), for the support and help rendered during plant collection and field work and also for being patient enough to sit through my field work.

"Your friend is your needs answered… And in the sweetness of friendship let there be laughter, and sharing of pleasures. For in the dew of little things the heart finds its morning and is refreshed" (Khaleel Gibran). Any words of acknowledgement are incomplete without the mention of friends who make this world a better place to live. I thank Mr. Farhan Maloof for helping me be levelheaded, throughout this process and for listening to me. A special word of thanks also goes to Mr. Arun Sathya C.V. and Mr. Nipun Kumar for being there whenever I needed them. I am forever obliged

to Mr. Praveen Jose for the love and emotional support he so freely gave, whenever I needed it.

I am forever grateful to Ms. Neethu K., Ms. Farhad V.P., Ms. Surekha Y. Pawar and Mr. Manu Philip for making my journey as memorable as possible. It is with your companionship that each day became a day to remember. There are miles to go before we rest. I am grateful to Mr. Aneesh S. and Ms. Sajidas for their support and consideration.

I am thankful to my sister Ms. Vinitha Viswanathan for always looking out for me. Growing up, you were my benchmark. Special thanks to Kannan and Aami for being the curious little beings who question everything I do. Your never ending questions and comments has always provided a fresh perspective to look at things.

I am forever indebted to my Amma and Achan, for letting me be who I am and for never setting boundaries. Any words of gratitude fall short of what you are to me. Thank you for just being the best. Amma, you deserve much admiration for the endurance you have shown in life in general and during my fieldwork in particular. I owe you much for the endless hours you spent, sitting among the shrubbery, at random places waiting for me to finish my work. I dedicate this thesis to you and Achan.

Finally I take this opportunity to express my gratitude to the almighty for the miracles, love, kindness and patience in my life. It is with you that I find my salvation.

Veena V.

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1. INTRODUCTION

Biologically, the basic aims of a species are self-preservation and procreation. Organisms thus have evolved various ways of reproduction, including sexual and asexual means in order to ensure procreation and, in turn self-preservation. In plants with diverse mechanisms to ensure vegetative reproduction, sexual reproduction act as a ploy in creating variability and ensuring survival at the species level. Pollination, being a key process for sexual reproduction, plays a vital role in the continued evolution of plants. Data from pollination biology add to our perception of the evolutionary process of reproductive isolation and speciation as well help in designing conservation plans (Sipes & Tepedino, 1995; Anderson *et al*., 2001). Mechanisms of sexual reproduction and pollination biology have drawn the attention of both commoners and naturalists from time immemorial. Though the female reproductive structures in plants were quite conspicuous and palpable, the plant equivalent of sperms eluded the pioneers in this field and remained a mystery, causing considerable dilemma among scholars and scientists. Historically, the earliest known recognition of sex in plants has been attributed to the ancient Assyrians. Archeological evidences suggest that they practiced hand pollination of the date palm. It took the invention of microscope for modern science to make any significant steps in recognizing the respective roles played by pollen grains and ovules in the sexual reproduction of plants. Pollination thus has been an area of interest from time immemorial.

It is the works of Kolreuter (1761) and Sprengel (1793), that provided detailed information about the reproductive processes in plants from pollination to fertilization. Sprengel (*l.c*.) described the floral adaptations in

many flowering species and concluded that the flowers have traits that attract insects and aid in pollination. Darwin further used this information to support his theories about the effects of inbreeding (1876), co-evolution (1862), and the contrivances for outbreeding in plants (1877a). Darwin thus had a crucial role in the evolution of the branch of plant reproductive biology (Pannell, 2009). Later, Darwin's work on floral structure and pollination inspired others to study the reproductive biology and the interaction between flowers and pollinators.

The study of reproductive biology of a plant involves the study of all aspects of reproductive events and their interactions with the environment. It thus encompasses the study of phenology, floral biology, interaction with the pollinators, breeding system, etc.

Living organisms, in general, show cyclic and seasonal patterns or responses to their environments. Such responses, generally termed phenology plays an important part in their reproductive success. Detailed investigations on the phenology of populations and communities help us to establish an understanding of the species specific adaptations to environmental conditions. Phenological studies are also important for understanding the plant-animal interactions. Reproductive phenol-events are most sensitive to climatic variations and an accurate knowledge of the relative shift in such events will undoubtedly help in predicting future changes (Shivanna & Tandon, 2014). Plants also optimize resource allocation to different physiologically active sites competing for resources (Alvim, 1964), and thus flowering may occur only when the availability of resources are not limited. Pollinators or dispersers may also act as limiting resources and the competing plants may evolve staggered flowering and fruiting phenologies (Levin & Anderson, 1970).

Despite recent criticisms, the idea that floral evolution is associated with pollination syndromes is still highly prevalent among biologists. Regardless of the association with pollination syndromes floral morphology is one of the most significant aspects of plant-pollinator interactions and in turn reproductive biology. Floral structure determines pollinator accessibility to floral rewards, pollen deposition on the pollinator, pollen transfer to the stigma, etc.

Individual species may show variations even in the longevity of flowers and could play a significant role in determining the interactions with pollinators (Primack, 1985). The evolution of floral structure is mainly thought to be driven by mechanisms of pollination. It is widely accepted that primitive plant families were bisexual and evolved mechanisms such as dichogamy to reduce self-pollination (Endress, 2010) and flowers with tubular corolla evolved from flat flowers in association with long tongued pollinators (Nilsson, 1988). Even the evolution of inflorescences from solitary flowers is thought to be influenced by pollinator behaviour as a cluster of flowers may not only reduce the risk of damage, but may also increase visits by pollinators.

Darwin (1877b) suggested that pollen size was related to style length while Cruden (1977) elucidated that the pollen to ovule (P/O) ratio indicated breeding systems in action. Even the occurrence of inbreeding in flowering plants has been suggested to be accompanied by a reduction in pollen production per flower (Vasek & Weng, 1988). The pollen stigma interactions are in turn depended on the viability of pollen grains, receptivity of the stigma and the genetic compatibility between the two (Dafni *et al*., 2005).

Pollination, the transfer of pollen grains from the anther to the stigma is one of the most important events in sexual reproduction of angiosperms. Based on the origin of pollen, pollination can be classified as autogamous (pollen transfer from anther to stigma of the same flower), geitonogamous (pollen

transfer from anther to stigma of different flowers of the same plant) and xenogamous (pollen transfer from anther to stigma of flowers of different plants) (Shivanna & Tandon, 2014). Both geitonogamous and xenogamous pollination require the aid or assistance of agents of pollination.

Insect pollinators represent a large proportion of the biotic agents of pollination. Angiosperms, being the latest species to evolve, are the most successful group of plants to occupy the planet and their evolutionary success has greatly been attributed to the evolution of flowers and the associated evolution of biotic pollinators (Pellmyr, 2002; Shivanna, 2003; Willmer, 2011). Pollinating agents bring huge ecological and economic benefits to flowering plants, wildlife and humans. Such pollinators are attracted by plants with the aid of visual attractants like colour and structure or floral scents or by the offer of rewards like pollen and nectar. The mostly mutualistic interactions between the pollinators and plants involve exchange of resources of the plant with the services of the pollinator (Ollerton, 2006). Understanding that not all visitors to a flower are pollinators is crucial to distinguish between the casual visitors, reward robbers and actual pollinators. The number and the frequency of visits often play an important role in determining the pollination efficiency.

Any decline in pollinator service will directly influence reproductive output, reducing the quantity and/or quality of fruit and seed set and also could promote selfing in self-compatible species (Rodriguez-Perez, 2005). The sustenance of plant populations is significantly influenced by both demographic (reproductive success) and genetic (inbreeding depression, evolutionary potential) mechanisms (Frankham & Ralls, 1998; Saccheri *et al*., 1998). The breeding system has considerable impact in determining the demographic and genetic parameters, and thereby influencing the population health and maintenance (Gaudeul & Till-Bottraud, 2004).

Pollination biology was developed as a descriptive science over many decades. Sprengel (1793) concluded that the flowers have adaptations to attract insects for pollination. This view that the morphological, chemical and other floral traits were evolved as adaptations to specific pollinators, was followed by the likes of Delpino (1873–1874), Faegri and Van der Pijl (1979), etc. After years and years of empirical research, presently, pollination syndromes are considered merely as a possible clue from which to start the exploration on floral biology. Biologists are now testing evolutionary and ecological hypothesis in pollination systems and are using this field of study to develop generalizable concepts.

Reproductive biology of plants was also indispensable for the initiation of genetics. Beginning from the time of Darwin, plant geneticists regularly use specific reproductive systems to examine the genetic basis of traits. By the end of the last millennium a new age has begun that merge ecological and genetic approaches to study the adaptive significance of reproductive traits of plants (Barrett & Harder, 1996). Wide neutral genetic variations are employed to discern the success of reproductive strategies of plants at both population and individual levels (Morgan & Conner, 2001). Presently, greater priority is given for establishing the genetic basis of plant reproductive traits with continuous variations such as pollinator attractiveness (Schemske & Bradshaw, 1999; Whittall *et al*., 2006).

Thus, deciphering plant reproductive mechanisms is of immense importance to various fields of science including conservation of biodiversity and the control of invasive species. It is considered that a knowledge of plant-pollinator interactions in plant communities in the tropics are far more important than elsewhere because of the increased threat to these eco-regions (Bawa *et al*., 1990). An understanding of the relationships between floral characters and floral visitors are most essential in studying the complexity,

interactions and influence of diverse organisms with plant communities and the distribution of rare plant species (Richard *et al*., 2011). In the last few decades, though the enthusiasm for the field of reproductive biology has hugely increased, groundbreaking studies in this field have been very limited all over the world and in India in particular. Much of the focus has been on the floral biology and plant-pollinator interactions of endemic, rare, endangered and threatened tropical and temperate species (Gaston, 1994) while most of the invasive species as well as those with no apparent ecological or economic significance are left untouched. This is very disappointing; especially with regard to the rich and diverse flora, the country has, over a wide range of habitats. There are 20,141 taxa of angiosperms under 2991 genera and 251 families in India, representing approximately 7% of the described species in the world (Karthikeyan, 2009). India is further characterised by high endemism, next only to Australia (Arisdason & Lakshminarasimhan, 2016). Many angiosperm families including Poaceae, Orchidaceae, Asteraceae, Euphorbiaceae, Commelinaceae, Lamiaceae, etc., with wide range of distribution all over the world are well represented in India. A thorough exploration of the pollination biology of the members of these families is much needed. It is in this context, the pollination biology of selected taxa of Commelinaceae has been undertaken.

1.1 Commelinaceae

Commelinaceae are a monocot family, popularly called as the ‘dayflower family’ or the ‘spiderwort family’, comprising about 41 genera and 734 species (Govaerts & Faden, 2015). The family shows a wide range of distribution, especially in the tropics, sub-tropics and warm temperate regions. Plants belonging to this family are found in diverse ecological habitats but grow primarily in humid, mesic forests and grasslands. Many species within this family find their use as an ornamental across the globe. A few are used as

vegetable or as a medicine in some parts of the world especially in the South Asian countries whereas some are considered as a threatening weed.

Commelinaceae comprise annual or perennial, terrestrial, rarely aquatic herbs with erect to ascending, spreading, decumbent or scandent shoots. The most peculiar features of their flowers are their short lives and lack of nectar. Flowers are arranged on terminal or terminal and axillary thyrses, commonly a panicle of helicoid cymes (cincinni). They are actinomorphic or zygomorphic, trimerous to pentacyclic with six stamens arranged in two whorls. About 97% of Commelinaceae possess either monomorphic bisexual flowers alone or bisexual and male flowers together while the remaining are either monoecious, gynomonoecious or polygamomonoecious (Faden, 2000). These plants are known to be autogamous or entomophilous and are renowned to use floral deception as a mechanism to attract pollinators (Faden, *l.c*.). With highly evolved mechanisms to ensure survival and adaptations to enhance reproductive potential with minimal expenditure of resources, these species forms one of the ideal systems to study reproductive biology.

Faden and Hunt (1991) recognized two sub families under Commelinaceae: Cartonematoideae (Pichon) Faden ex G.Tucker and Commelinoideae Faden & D.R. Hunt, based on the presence of glandular microhairs, raphide canals, etc. The subfam. Commelinoideae is further classified into two tribes, namely Tradescantieae and Commelineae. Members of the tribe Commelineae are distinguished by their almost cosmopolitan distribution, absence of silica in epidermal cells, symmetry of flowers, etc. (Faden, 1998). *Commelina diffusa*, *Dictyospermum montanum*, *Floscopa scandens*, *Murdannia nudiflora* and *Rhopalephora scaberrima* adequately represent a wide range of the characteristics of the family and particularly of the tribe Commelineae. They are found extensively in Kerala distributed in varying habitats from deep forests to road sides.

1.1.1 *Commelina diffusa* Burm.f.

Commelina, the largest genus in the family Commelinaceae comprises about 70 species (Faden 2006, Sheba & Nampy, 2012b), and are known as day flowers due the short lives of their flowers. The genus is represented by 25 species in India (Sheba & Nampy, 2012b). The day flowers are annual or perennial herbs, characterized by their zygomorphic flowers born in terminal and leaf-opposed cymes enclosed in folded spathes (Deyuan & DeFilipps, 2000). *Commelina diffusa*, popularly known as 'the climbing or spreading dayflower' is distributed throughout the tropical regions of America, Africa, Asia and the Pacific, and in the subtropical regions of southern USA, South America, Australia and South Asian islands (Holm *et al*., 1977). It is widely used as a medicine in many South Asian countries including China and the North-Eastern states of India (Prima *et al*., 2019). The leaves are diuretic and febrifuge, wounds are poulticed with the mucilage from the stems, and the plant extracts are also used in the treatment of high blood pressure and bladder infection (Corrigan *et al*., 2011). The antioxidant, anti-inflammatory (Mensah *et al*., 2014), antibacterial, antifungal (Oulowagbenga, 2017), nephro and hepato-protective properties of *C. diffusa* has been scientifically explored and established.

Because of vigorous nature of growth, forming dense strands, *C. diffusa* tends to compete with low growing crops such as vegetables, pulses, cereals, pasture grasses, etc., and smother them (Holm *et al*., 1977; Issac *et al.,* 2013). It is considered to be a major problem weed for 17 crops in 26 countries, where they became persistent and difficult to manage (Holm *et al*., 1977). *C. diffusa* grows well in cultivated soils of cocoa (*Theobroma cacao*), citrus, water tolerant root crops such as *Colocasia esculenta*, and is also a major weed in sugarcane (*Saccharum officinarum*), rice (*Oryza sativa*), soybean (*Glycine max*), cassava (*Manihot esculenta*), corn (*Zea mays*) and plantain

(*Musa* spp.) (Holm *et al*., 1977). As it is a broad leaved weed it is generally not considered highly competitive for nutrients but their weedy or allelopathic potential is not well studied and needs to be ascertained.

1.1.2 *Dictyospermum montanum* Wight

Dictyospermum Wight, is a comparatively small genus represented by four or five species in the world (Deyuan & DeFilipps, 2000), of which three are found in India, *viz*. *D. montanum*, *D. ovalifolium* and *D. ovatum.* They are perennial herbs with erect to decumbent shoots and leaves spirally arranged, often clustered toward the apex of the stem. *D. montanum*, commonly called 'mountain day flower', is distributed in South India, Sri Lanka, Assam and Indo-China. It grows along the borders and within forest areas or lining ghat regions. Their ability to adapt and survive while spreading to larger areas is typical of the family.

1.1.3 *Floscopa scandens* Lour.

Floscopa Lour., comprising of about 20 species (Deyuan & DeFilipps, 2000), is mostly aquatic or semi-aquatic genus that are widely distributed in tropical and subtropical areas. It is represented by a single species in India, *F. scandens*, commonly called the 'climbing flower cup'. They are much branched, decumbent perennial herbs with cauline leaves and inflorescences in terminal or axillary thyrses. These are commonly used as aquarium plants and are gaining popularity around the world. This species also finds its use medicinally as a febrifuge and also for relieving pyodermas, abscesses, and acute nephritis (Deyuan & DeFilipps, 2000).

1.1.4 *Murdannia nudiflora* (L.) Brenan

Murdannia Royle, the fourth largest genus of the family, is represented by 26 species in India (Ancy & Nampy, 2015). The genus comprises of annual or

perennial herbs distributed widely in the tropics and limitedly in the temperate regions. *M. nudiflora*, commonly called as the 'dove weed' is distributed form the tropical and subtropical Asia to South-West Pacific. The plant is widely used in folk medicines in the North-Eastern regions of India,China, Caribbean and Trinidad (available from: http://www.stuartxchange.com/Alikbangon.html) in the treatment of asthma, leprosy, stomach ailments, etc. While the root paste mixed with goat milk is prescribed orally for asthma, a paste of the whole plant with common salt is used to cure leprosy. Patwari *et al*. (2014) demonstrated significant analgesic activity in *M. nudiflora*, comparable to a standard drug, diclofenac sodium and established the need for further exploration towards the medicinal potential of these plants.

Murdannia nudiflora is a potential weed and it infests about 16 different crop species in 23 countries (Holm *et al*., 1977). It is a troublesome weed for rice (*Oryza sativa),* groundnuts (*Arachis hypogea),* cotton (*Gossypium arboreum*), soybean (*Glycine max*), etc. (Soerdersan *et al*., 1974; Moody, 1989; Bastidas-Lopez, 1996; Wilson *et al*., 2006). *M. nudiflora* is considered an aggressive weed that can out-compete desirable species for light and nutrients and replace them (Holm *et al*., 1977; Atkinson, 2014).

1.1. 5 *Rhopalephora scaberrima* (Blume) Faden

Rhopalephora Hassk., first described in 1864 is represented by four species (Deyuan & DeFilipps, 2000) and distributed widely in the regions of Madagascar, tropical and subtropical Asia and the southwest Pacific. They are spreading perennial herbs with proximally creeping stems that become erect distally. The genus is represented by a single species, *R. scaberrima* in India. It is popularly known as the 'rough day flowers' and are seen growing profusely along the sides of streams or damp forest floors.

1.2 Importance of the present study

Commelinaceae, with worldwide distribution and extreme diversity in the tropics is represented by 14 genera and approximately 100 species in India. Studies on the reproductive biology of this family have been very limited all over the world and in India in particular. While internationally the likes of Owens (1981), McCollum *et al.* (1984), Simpson *et al.* (1986), Williams and Walker (2003), Hrycan and Davis (2005), Ushimaru *et al*. (2007), Oziegbe *et al*. (2013), Sigrist and Sazima (2015), etc., have worked on the reproductive biology of some species including *Commelina erecta*, *C. communis*, *C. coelestis*, *C. dianthifolia*, *Dichorisandra* sp., *Pollia crispata*, *Tinantia anomala,* etc., nationally there has only been the works from Kaul and Koul (2008, 2009, 2012) in *Commelina benghalensis* and *Commelina caroliniana.* A detailed knowledge of the reproductive aspects of the members of such a diverse group with such varying skills of adaptability will surely be enlightening, not only for the control/conservation of these species but also could be extrapolated to other vascular plants. This group thus forms an ideal framework to explore the reproductive and pollination potential for further academic as well as practical references. The five species selected serve to represent the characteristic features of the family in general and of the tribe in particular, and equip us with the preliminary ability to understand the pollination biology of the family. Thus this work is intended to add to and fortify our knowledge of the reproductive aspects of plants in general and specifically of those of the family Commelinaceae. All the major aspects of pollination biology, that are fundamental to the study, including phenology, floral biology, pollen and pistil biology, pollination ecology, breeding system, fruit and seed set and seed germination are covered for the selected species.

1.3 Objectives

1. To study the flowering phenology (flower number, type, structure, time of anthesis, anther dehiscence, etc.).
2. To study the floral biology (structure, sexual system and morphological adaptations of the flowers in relation to breeding system and pollination ecology).
3. To study the pollen and pistil biology (pollen morphology, pollen viability, stigma morphology, receptivity).
4. To identify the major pollinators and the mechanism of pollination.
5. To evaluate the reproductive efficiency.

2. REVIEW OF LITERATURE

2.1 A brief historical review of pollination biology

As civilizations began to take root so did the interest of humans in nature and natural phenomenon. The earliest known record of sexual reproduction in plants is attributed to ancient Assyrians. Several bas-reliefs were recovered from this ancient civilization that depicts the hand pollination of the date palm, *Phoenix dactylifera*, interlaced with mythological themes (Olmstead & Olmstead, 1923; Real, 1983).

The first empirical evidence that plants reproduce sexually is attributed to the German physician and botanist Rudolf Jakob Camerarius (1694). While studying some bisexual species, he noted that both stamens and pistils were needed for seed production. The details of fertilization were discovered by scientists several decades after Camerarius's death.

Another notable contribution came from J.G. Kolreuter (1761), who in his 'Verlaufiger Nachricht vom einigen das Geschlecht des Pflanzen betreffenden Versuchen und Beobachtungen', recognized the importance of insects in flower pollination. From his observations that many flowers had something attractive to the pollinators, he concluded that flowers which were unable to pollinate themselves were pollinated by insects. Kolreuters' contributions to the morphology of pollen grains, despite the lack of a proper microscope, were notable. He discovered the two distinct coats in the outer covering of pollen and also made a note of the orifices and their lids in the exine of pollen from *Passiflora*. The production of artificial hybrids of *Nicotiana, Mathiola, Hyoscyamus,* etc. is considered as Kolreuters' greatest contribution to the field of reproductive biology.

Christian Konrad Sprengel (1793) made remarkable observations not only on the sexuality of flowers but also on the purpose of elaborate floral hairs, nectar, coloured patterns on corolla, etc. He believed cross-pollination to be the rule rather than exception and wrote "nature appears not to have intended that any flower should be fertilized by its own pollen" (Woodhouse, 1935). Sprengel even made note of distinctions between anemophily and entomophily. Though Sprengel made unparalleled contributions to the field of pollination biology, his work remained unnoticed for a long time (Real, 1983). Müller (1883) suggested that Sprengels' failure in depicting the advantageous of cross-pollination might have been a reason for his works being overlooked.

Thomas Knight (1799) reported that the progenies of his hybridization experiments produced many new variants which were superior to their parents. He concluded that no plant self-fertilizes itself for the perpetuity of generations (Real, 1983). Giovani Amici (1830) demonstrated that the pollen grains germinating on a stigma sent pollen tubes down the style to the ovules. But it was John Smith in 1841, who disproved the popular belief that the 'seed' was in the pollen and that it was transplanted into the ovule after travelling down the pollen tube. Karl Friedrich Gartner's (1849) "Versuch und Beobachtungen uber die Bastardzeungung", provided an exhaustive review of sexual reproduction in plants.

Another prominent name in this field is that of Darwin (1877a) who published an important book on pollination, titled '*The various contrivances by which orchids are fertilized by insects'*. He established that many orchids had developed elaborate structures through natural selection to promote cross-pollination. He proposed that by interacting with each other over many generations, orchids and their pollinators have evolved together through co-evolution.

Henslow (1891) disregarded the benefits of cross-pollination and considered flower shapes and colours to be caused by the "irritation" of insect visitors. He made some noteworthy observations on the specificity of insect visitors and on the advantages of self-pollination in single propagules that are transported long distances.

Porsch (1909) travelled extensively to observe pollination mechanisms, especially ornithophilous ones. He was the first to address bat pollination as an important feature of tropical pollination mechanisms (Porsch, 1931).

The rise of neo-Darwinism also propelled pollination biology research. Studies on foraging patterns of visitors using sequences of photographs (Baker & Harris, 1957; Barker, 1970), night vision devices (Ayensu, 1974) radio telemetry (Fleming *et al*., 1977; Heithaus & Fleming, 1978) etc., gained considerable importance during this period.

In later years these studies have moved towards an ecosystem approach of pollination biology (Opler *et al*., 1980) and also their energetics (Kevan & Baker, 1983). Recent studies in this field explore the influence of pollinator driven adaptations and plant-pollinator systems in phylogeny (Ollerton, 1996; Johnson & Steiner, 2000, Van der Niet *et al*., 2014; Adderley & Vamosi, 2015; Jones & Agrawal, 2017).

2.2 Pollination biology of the family Commelinaceae

Commelinaceae is an herbaceous family of entomophilous or autogamous monocots with 41 genera and 734 species worldwide (Govaerts & Faden, 2015). Beatty and Beatty (1953) determined the duration of the stages in microspore development, from the end of meiosis, through the first microspore division, in *Tradescantia paludosa*. They described five morphological stages, from the end of the tetrad stage following meiosis to the first microspore division, with duration of about 24 hrs each. Their

exhaustive study revealed that it takes around 36 hrs to complete first microspore division, 24 of which are spent in very early prophase, 4.43 in early prophase, 3.23 in mid-prophase, 2.75 in late prophase, 0.36 in pro-metaphase, 0.50 in metaphase, 0.50 in anaphase and 0.23 in telophase.

Floral dimorphism is a common feature in the family Commelinaceae. Maheshwari and Maheshwari (1955) did the first reports on floral dimorphism in *Commelina forskalaei* and *C. benghalensis.*

Rowley (1959) investigated the physical structure of the pollen and pollen wall in 19 species representing 11 genera of Commelinaceae. They found that the basic structural elements of mature pollen wall in the Commelinaceae members studied were comparable and any variations in morphology at the microscopic level were due to the distinctly individual arrangement of those components.

Stevenson and Owens (1978) studied the reproductive morphology of *Gibasis venustula,* a self-incompatible species of Commelinaceae. They described the developmental anatomy of the flower with particular attention to the anther. They recorded anther wall of reduced-type, in Commelinaceae for the first time and found enhanced protein and RNA content in the tapetum and sporogenous tissue.

Poole and Hunt (1980) carried out an exploratory survey of the pollen morphology and taxonomy of the family Commelinaceae. They studied about 100 species belonging to 24 genera with a greater focus on *Tradescantia*. They found that the pollen was monosulcate except for three species: *Tinantia anomala* and *Zebrina pendula* with three germinal apertures and *Tinantia pringlei* with an extended sulcus. They observed four major types of tectum ornamentation each in the tribes Tradescantinae and Commelineae. For both tribes it was found that specialization in the pollen was accompanied by a

specialization in the androecium. The variation in pollen morphology as illustrated by this survey implies the potential of pollen characteristics in solving taxonomic problems at tribal and sub-tribal levels.

Owens (1981) evaluated self-incompatibility in selected taxa of the family Commelinaceae. Of the 110 species (representing 22 genera) studied, fifty-five were self-incompatible, fifty were self-compatible and the remaining five include both self-compatible and self-incompatible individuals. They found that the self-incompatible species possessed actinomorphic flowers and the majority of them belonged to the tribe Tradescantineae. Whereas those with zygomorphic flowers, more commonly found in the tribe Commelineae, were self-compatible. Pollen grains in all the species studied were of the binucleate type and the site of pollen tube arrests in self-incompatible species were found to be at or near the base of the stigma papillae, except in *Dichorisandra* sp. and *Siderasis fuscata*, where pollen tube-arrest was stylar.

Owens and Kimmins (1981) studied the stigmatic morphology of 68 species of 17 genera of Commelinaceae and found that the stigmas of majority of the species studied were either trifid or triangular and all of them were papillate. The stigmas in the genera of *Callisia*, *Tripogandra* and *Zebrina* were of the dry type while those in some species of *Aneilema* and *Commelina* showed wet stigmas. They also reported variations in the size, shape and composition of stigmatic papillae. In most species while the papillae covered the stigma completely, in *Coleotrype natalensis*, *Cyanotis* species and *Siderasis fuscata*, the papillae was only present around the neck of the stylar canal. They classified the papillae based on their shapes into three genera specific categories *viz*. A (*Aploleia*, *Callisia*), F (*Commelina*) and G (*Cyanotis*), one species specific category (category D specific to *Tradescantia fluminensis*), and three other categories, each present in different genera. In *Gibasis* and *Tradescantia* blisters of various sizes covered the stigmatic surfaces but these

were not found in *Callisia*, *Cyanotis*, *Aneilema* and *Commelina* species. The stigmatic surface papillae in *Aneilema* and *Commelina* were found to be covered in a cuticular fold.

Keighery (1982) reported facultative cleistogamy in *Murdannia nudiflora* and remarked that cleistogamous flowers were produced on rainy days and chasmogamous flowers on sunny days. During a period of 7 days, he observed no insect pollinators on the species.

Owens and Horsfield (1982) studied stigmas in *Aneilema* and *Commelina* species using light and electron microscopy. The stigmas of those species were reported to be trifid and to comprise elongated papillae. The papular cells of open flowers were recorded to show progressive degeneration. Fluid exuded from the hollow style was found to reach the surface, oozing through fissures in the cuticle at the inter-papillar junctions and into the interstices at maturity, making the stigmas "wet". Their results indicated that pollen attachment, hydration, germination and early tube growth were very rapid following self-pollination and that the pollen tubes got through the neck of the style within ten minutes of germination. They proposed the characteristic features of pollen and stigmas in these genera as an indication of monophyletic origin.

Owens and Dickinson in 1983 studied the intine and exine development of the pollen wall in *Gibasis karwinskyana* and *G. venustula*. They found that the appearance of electron-opaque depositions or tri-partite plates at discrete sites between the plasma membrane of the spore and the inward surface of the callose special wall during the tetrad stage was the first indication of exine development and that the exine pattern was clearly established within the tetrad. After the release of the spores from the tetrad, it was observed that an intimate association is rapidly developed between the plasma membrane of the periplasmodial tapetum and the newly-formed exine. Compacted electron-

opaque material was found at the interface between the membrane and the exine and vesicular material is added from the tapetum. Intine development was reported to begin just before pollen grain mitosis and intine deposition to take place after mitosis; a bilayer was apparent in mature grains. They also found that the exine of the mature spore stains less intensely than in the young spore and that the interbacula spaces were filled with material from the degenerated tapetum.

Zavada (1983) executed a study of the evolutionary trends in apertures and wall structures of monocot pollen grains. They reported the pollen in Commelinaceae to be predominantly monosulcate and triaperturate in *Commelinanta* and *Tinantia*. Pollen wall structure of 11 taxa has also been reported in this study and found that the exine sculpturing varies considerably in these genera.

Owens *et al*. (1984) studied the anatomy, histochemistry and ultrastructure of the stigmas and styles in 37 species of 13 genera of Commelinaceae. They found that the stigmas in Commelinaceae were papillate and that in most species the papillae formed a dense fringe of cells around the mouth of the stylar canal. It was reported that the style in Commelinaceae comprised an epidermis, cortex and a hollow tripartite canal leading to the ovarian cavity, except in *Aploleia multiflora*, in which the style is solid and the transmitting tissue comprises electron opaque cells. They did not find any relation between the occurrence of incompatibility and stigmatic structure.

McCollum *et al.* (1984) investigated the reproductive biology of *Commelina erecta*. They reported delayed selfing and autogamy in this species and the effect of pollination in the first formed flowers on the fruiting of the subsequent flowers within a spathe.

Simpson *et al*. (1986) studied the reproductive biology of *Tinantia anomala* and reported that *T. anomala* exhibits a pollen mimicry system which promotes cross-pollination while reducing pollen loss to foragers. Their observations and experiments revealed that *T. anomala* is predominantly autogamous and that there is little visitation from native insects that could fall for pollen mimicry. They showed that the low seed set in natural populations was due to resource limitation as opposed to the automatic abortion of carpels as suggested by Torrey (1859).

Faden (1992) studied the role of floral hairs in floral attraction in the members of Commelinaceae and found that the occurrence of floral hairs is very common in the family and the hairs related to pollination are generally found associated with the androecium. According to him (*l.c.*), their functions might include attracting insects to the flowers, providing footholds, retaining fallen pollen and determining insect behaviour on the flowers.

Faden (1998) in 'Families and Genera of Vascular Plants' provided the general features of the family Commelinaceae, with focus on floral and inflorescence morphology. He noted that the zygomorphic nature of the flowers in this family was solely due to the nature of the androecium. According to him, the occurrence of floral dimorphism in various forms such as male and bisexual flowers, cleistogamous and chasmogamous flowers, enantiostylous flowers etc. to be prevalent among the members. Variations within the androecium and the arrangement of dimorphic anthers for various genera within the family were also described by Faden (*l.c*)

Faden (2000) also carried out a detailed study of the floral biology of Commelinaceae. He described the floral morphology with emphasis on characteristics such as sexuality and floral dimorphism, phenology, floral deception, etc., across the family. He found that both bisexuality and andromonoecy were common in the family whereas monoecy, gynomonoecy,

and polygamomonoecy were rare. He established that different 'sexed' flowers might open at different times in *Aneilema umbrosum* subsp. *umbrosum* and in several species of *Palisota*. He also reported floral deception to be common and often to be found in association with heteranthery.

Kaul *et al*. (2002) investigated the reproductive effort and sex allocation strategy in *Commelina bengalensis*. They reported that in *C. bengalensis* three types of branches (negatively geotropic, positively geotropic and diageotropic) and four types of flowers (unisexual or bisexual, chasmogamous and cleistogamous) are present. According to them, pollen/ovule ratio, female:male biomass ratio and reproductive output vary between different flower and branch types.

Williams and Walker (2003) studied the pollination in *Pollia crispata* and reported syrphid flies, halictid bees and apid bees as the major pollinators of this species. According to them though *Pollia* is an entomophilous species it can reproduce vegetatively and also produce viable seeds without the aid of insects.

Ushimaru *et al*. (2003a) studied the sizes of various floral organs of *Commelina communis* to test the hypothesis that size correlations among floral organs are regulated by natural selection. They evaluated the size correlations among both perfect and male flowers. Their studies revealed that in perfect flowers the stigmas might have evolved to be positioned so as to optimize the transfer of pollen from anthers.

Ushimaru *et al*. (2003b) also tested the regulative effect of pollinator-mediated selection in the size variations of floral organs. Their investigations revealed that the style length in perfect flowers might have been stabilized

through pollinator mediated selection the size of attraction related organs vary more than the size of mating related organs.

In 2005 Hrycan and Davis studied and compared the structure and pollen production of the stamens and staminodes in *Commelina coelestis* and *C. dianthifolia*. They found that both the species studied were entomophilous and facultatively autogamous. Their studies revealed that pollen from the lateral stamen was important for cross-pollination and that from the central stamen act as a reward for pollinators and also for delayed autogamy.

Ushimaru *et al*. (2007) studied the influence of coloured floral organs on pollinator behaviour and pollen transfer in *Commelina communis*. They demonstrated that both the non-rewarding and rewarding (yellow) anthers made significant contributions in directing insect visitors towards optimal landing positions and also in increasing the number of floral visitors. They also established that the absence of rewarding anthers decreased the removal of pollen from the other anthers and also the reception at stigmas.

Kaul and Koul (2008) studied the floral phenology of *Commelina caroliniana* in relation to pollination and reproductive output. They opined that the flowers were structured for cross-pollination and that the weak protandry they observed also suggests cross-pollination. They suggest that in the situations where cross-pollination failed, selfing took over.

Hardy *et al*. (2009) investigated the floral organogenesis and the developmental basis for pollinator deception in *Commelina communis*. They also studied the developmental retardation in the gynoecium in correspondence with the staminode development in the upper half of *C. cummunis* flowers. They summarized that such retardations are unlikely to be found in functionally fertile organs like stamens and ovule-producing carpels, as key preparatory events preceding sporogenesis could otherwise be

disturbed. They also concluded that the differential growth about the floral axis resembles that in some eudicots, known to be regulated by the TCP gene family.

Kaul and Koul (2009) studied sex expression and breeding strategies in *Commelina benghalensis* and found that in the majority of chasmogamous flowers, the male and female reproductive phases overlap, facilitating self-pollination. They reported exceptions to this general rule where, in the chasmogamous flowers the female phase matures either prior to anther dehiscence or in flowers in which anthers are sterile, thereby facilitating cross-pollination. They concluded that in *C. benghalensis* a mixed mating strategy exists where the cleistogamous flowers contribute to the production of selfed seeds and the chasmogamous ones produce outcrossed progeny.

Ushimaru *et al.* (2009) investigated the effect of flower orientation on pollen transfer in bilaterally symmetrical flowers. They reported a high fitness rate in horizontally oriented zygomorphic flowers, as they demonstrated increase in pollen transferr in zygomorphic flowers with horizontal orientation. This flower orientation is thus considered to enhance pollinator recognition and impact landing positions.

Panigo *et al*. (2011) discussed the inflorescence structure in the family Commelinaceae. He described the inflorescence to have an indeterminate axis (polytelic). He claims that variations found among the inflorescence types were due to the difference in the number of cincinni or due to the number of flowers.

Sheba and Nampy (2012b) revised the genus *Commelina* in India along with a morphometric analysis. They carried out a cluster analysis using 52 characters for 25 species in India. The 25 species were categorized into 6 distinct clusters in 2 sections.

Kaul and Koul (2012) investigated the staminal variations and their possible significance in *Commelina benghalensis* and *C. caroliniana*. They considered the yellow staminodes to provide excellent contrast with the blue corolla, thus being attractive to a variety of insects. They also found that the viability of the staminode pollen was also significantly lower.

Sheba and Nampy (2012a) discussed the importance of capsule and seed morphology by undertaking a light microscopic study on the capsules and SEM studies on the seeds of 22 species of the genus *Commelina* occurring in India. They assembled data on the size, shape and the number of capsules within a spathe and the number, shape, size, colour and ornamentation of testa of the seeds. They suggest that within this group, the capsule and seed characters are as one of the most useful tools in the species level classification.

Oziegbe *et al*. (2013) evaluated the role of stamens and breeding system in three selected species of *Commelina*. They found that in the species studied though the pollen from the lateral and central stamens set seeds, the pollen from the staminodes rarely set any seeds. It was also observed that the stigma faced the central anther while the style coiled in all three species.

Ushimaru *et al*. (2014) studied whether urbanization promote floral diversification with reference to pollinator availability. They tested their hypothesis in *Commelina communis* and examined whether floral traits such as the petal length, degree of herkogamy, P:O ratio, and staminate flower production changed with pollinator availability across the urban-rural gradient. Their results suggest that factors affecting or causing phenotypic variations were formed as a result of urbanization.

In 2015, Rubin studied visual attraction and buzz pollination by anthophorid bees in *Coleotrype madagascarica*. They found that the flowers attracted

several species of pollen seeking insects and that their pollination relies on female bees that buzz the bright yellow staminal filament hairs and deposit pollen on the stigmas of hook-shaped styles in other flowers. They found that anthorphid bees practicing buzz mechanism were the most frequent visitors and the most apparent pollinators

Sigrist and Sazima (2015) studied *Dichorisandra* sp. The major focus of the study was on the diversity, phenology and reproductive biology of *Dichorisandra* along with the breeding system and the performance of buzzing bees other pollinators. Their studies showed that besides moisture, photoperiod also affects the flowering of some species. *Dichorisandra* were found to have zygomorphic flowers with poricidal, oligandrous anthers. They were mainly pollinated by Apid and/or Halictid bees using buzz mechanism.

Sripathy *et al*. (2016) carried out a study of the comparative seed morphology of four taxa of the genus *Commelina*, viz. *C. benghalensis, C. auriculata*, *C. diffusa* and *C. forskaolii*. Their studies indicated that seed surface ornamentation as a reliable tool for taxonomic deliniation.

Veena and Nampy (2019) studied the occurrence of inducd cleistogamy as a strategy for reproductive assurance in *Murdannia nudiflora.* They reported the occurence of autogamy and the production of cleistogamous flowers in this species as a mechanism to ensure reproductive success when pollinators are insufficient or when environmental conditions are unfavourable. Humidity and precipitation rates were found to be positively correlated with the number of cleistogamous flowers produced and the temperature as negatively correlated with the number of cleistogamous flowers. They reported that, vegetative reproduction, entomophily, and cleistogamous self-pollination together help this species to achieve optimal propagation.

Salamma *et al*. (2019) explored the pollen morphology of 15 species in Commelinaceae, including 6 species from *Commelina*, 7 from *Cyanotis* and 2 from *Murdannia.* They conclude that although these species share features such as heteropolarity and monosulcate aperture, they show diversity in size, shape and ornamentation and these features are considered to be of great importance in taxonomical, melissopalynological and aeropalynological studies.

Bose and Paria (2019) investigated the seedling morphology of eight species belonging to four genera of the family Commelinacceae using light and scanning electron microscopy. They documented the characteristics of the seed (shape, surface, hilum etc.), germination pattern (cotyledon, cotyledonary hyperphyll or apocole, cotyledonary hypophyll or cotyledonary sheath, hypocotyl etc.), root, first leaf etc. They employed multivariate analysis to explore the affinity of the investigated taxa supports and found that some of the relationships inferred were supported by previous studies involving pollen and floral morphology, DNA (rbc-L, 5S NTS, trnL-trnF) analysis etc.

Veena and Nampy (2020) studied the role of dimorphic anthers in *Commelina diffusa*, *Dictyospermum montanum* and *Rhopalephora scaberrima.* They confirmed the 'division of labour hypothesis' and found that one set of anthers infatuate the insects and the other maximize pollen exports utilising the 'safe-sites' on the body of the insects.

3. AREA OF STUDY

The present study was conducted in selected locations across Kerala where populations of chosen species were naturally growing (Fig. 1). Kerala, the southernmost state of India, lies between 8°18'–12°48' N and 74°52'–77°22' E. The state with a total area of 38,863 km^2 is bordered by the states of Karnataka and Tamil Nadu to the North, North-East and South and by the Arabian Sea to the West. Extending from the North to South on the East is the great natural barrier of the Western Ghats. A warm-humid climate with mean daily temperatures ranging from 19.8° to 37°C, perennial water resources, nutrient-rich soil etc., has attributed to the diverse vegetation and enormous species diversity in the state.

Geographically the state is divided into three regions *viz*., highlands, midlands and lowlands. The high lands include the Western Ghats and the valleys comprising dense evergreen forests while the midlands are the areas between the mountains and low lands, including small hills and uneven terrains and the lowlands include the coastal regions of the Arabian Sea.

Kerala receives both the South-West and North-East Monsoons and is responsible for the tropical humid climate with intermittent dry periods. Generally rainy season in the state begins by early June and extends to mid-December with an interval during September-October. About 10,000 km^2 land area of Kerala is home to major types of forests including wet evergreen and semi-evergreen forests, tropical moist deciduous forests, tropical dry deciduous forests, montane subtropical and temperate shola forests and grasslands (Thomas, 2000).

- Each species has been studied at two different locations and has also been introduced and monitored at the Calicut University Botanical

Garden. The sites chosen for the study include Vellimadukunnu, Kakkayam, Adivaram (Kozhikode district), Calicut University Campus (Malappuram district) and Vellanippacha (Thrissur district).

Vellanippacha is a hilly evergreen to semi-evergreen forest coming under the Pattikkad range of the Thrissur forest division. The forest area shows much degradation due to human interference. Kakkayam, at 2500 m above sea level, comes under the Peruvannamoozhy Forest Range, and is included in the areas marked for the Malabar Wild Life Sanctuary. The Kakkayam region encloses wet- evergreen to semi evergreen forests infested with many seasonal and permanent streams and rivers. Vellimadukunnu is a small human inhabited area within Kozhikode district. The area is largely urbanized but the vegetation flourishes along the Punur River. Calicut University Campus is situated in Malappuram district. The campus is situated in a lateritic hilly area with the vegetation of a scrub jungle. Adivaram comes under the political boundary Kozhikde district and is 11 m above sea level. It is an area along the Thamarasseri ghat road to Wayanad district and the vegetation is mixed with teak and pineapple plantations.

3.1 *Commelina diffusa*

Natural populations of *C. diffusa* growing at Vellimadukunnu (Kozhikode district, 11^{0}18.1072'N 75^{0}48.9254E) and Calicut University Campus (Malappuram district, 11^{0}7.9851'N 75^{0}53.3720'E) were identified. Each population was spread over an approximate area of 2 × 2 m. At Vellimadukunnu, the selected population inhabited an open patch of land exposed to sunlight. The temperature at the area was recorded to be in the range of 24.6–36.7 ^{0}C and the relative humidity was between 79–93%. At the Calicut University Campus, *C. diffusa* populations were growing in areas with lateritic rocks. There the temperature ranged from 30–38^{0}C and relative humidity ranged from 69–88%.

3.2 *Dictyospermum montanum*

Natural populations of *D. montanum* growing at Kakkayam (Kozhikode district, 11.5488°N, 75.9266°E) and Vellanippacha (Thrissur district, 10.58871667^{0}N 76.339225^{0}E) were identified. The population at Vellanippacha was growing in mass and covered an area of 3–4 m wide along the length of a stream, of which an area of 5 × 3 m was marked off for the study. At Kakkayam, *D. montanum* grew in small patches, covering a relatively smaller area. The population at Vellanippacha was growing deep within the hilly forest along the side of a stream. The average temperature ranged from 26–30^{0}C and the relative humidity ranged from 69–87%. At Kakkayam the plants grow along the ghat sides intermingled with other plants. The average temperature ranged from 21–31^{0}C and the relative humidity ranged from 70–85%.

3.3 *Floscopa scandens*

Natural populations of *F. scandens* growing at Adivaram (Kozhikode district, 11^{0}29.0720'N 76^{0}0.7290'E) and Kakkayam (Kozhikode district, 11.5487°N, 75.9264°E) were selected for the study. At Adivaram the plants grew spreading along the sides of a stream and flourishing in areas where it was exposed to direct sunlight. 27–35^{0}C of temperature and 63–79% of relative humidity was recorded. At Kakkayam, the population was found spreading within a swamp inside the forest and the temperature ranged from 21–31^{0}C and the relative humidity ranged from 70–85%.

3.4 *Murdannia nudiflora*

Murdannia nudiflora populations growing naturally in the Calicut University Botanical Garden, Malappuram district, 11^{0}7.9850'N 75^{0}53.3710'E) and at Vellimmadukunnu Kozhikode district, 11^{0}18.1070'N 75^{0}48.9250E) were identified and selected as the study site. The population at CUBG was spread

across 3 × 3 m area and that at Vellimadukunnu was about 1 × 1 m in area. At the Calicut University Campus, *M. nudiflora* was growing in loose soil exposed to direct sunlight. The temperature ranged from 30–38^{0}C and 69–88% and at Vellimadukunnu, these plants were growing in wet soil near drainage. The temperature and relative humidity at the area was recorded to be in the range of 24.6–36.7 ^{0}C and 79–93% respectively.

3.5 *Rhopalephora scaberrima*

Natural populations of *R. scaberrima* growing at Adivaram (Kozhikode district, 11^{0}29.0720'N 76^{0}0.7290'E) and Vellanippacha (Thrissur district, 10.5887^{0}N 76.3392^{0}E) were identified. At both locations the plants grew spreading along the sides of streams and 3 × 4 m areas were marked out for the study purpose at each locality. At Adivaram the plants grew along a slope on the side of the ghat road and flourished both under shade and exposed to sun. Temperature 27–32^{0}C and 69–82% of relative humidity was recorded at Vellanippacha the plants were growing atop a rocky hill within the forest along the side of a stream. The average temperature ranged from 26–30^{0}C and the relative humidity ranged from 69–87%.

4. MATERIALS AND METHODS

Five species of Commelinaceae, viz. *Commelina diffusa* Burm.f., *Dictyospermum montanum* Wight, *Floscopa scandens* Lour., *Murdannia nudiflora* (L.) Brenan and *Rhopalephora scaberrima* (Blume) Faden were chosen for the study. The study was conducted on plants maintained in Calicut University Botanical Garden and at two natural habitats for *Dictyospermum montanum*, *Floscopa scandens* and *Rhopalephora scaberrima* (Blume) and at two natural populations, including those at the Calicut University Botanical Garden, for *Commelina diffusa* and *Murdannia nudiflora* (Fig. 2).

4.1 Description

Detailed morphological description of the plants and flowers were prepared using the terminology of Faden (2000) and Simpson (2006). Photographs of floral parts were taken with a Stemi 508 stereomicroscope (Zeiss, Oberkochen, Germany) and EOS 7D and EOS 700D DSLR camera (Canon, Japan).

4.2 Phenology

The responses of the selected species to seasonal changes and the cyclic changes within the individuals were studied.

4.2 1 Population phenology

Phenological studies were carried out through regular field visits during the entire flowering seasons in 2015–2019. The following major pheno-events were recorded for the plants in the study population.

- Initiation of germination.
- Initiation of inflorescence and floral buds.

- Initiation, duration and intensity of flowering.
- Floral phenology (anthesis and post anthesis changes).
- Initiation and duration of fruit setting.
- Maturation of seeds.
- Details of seed dispersal.
- Perishing.

4.2.2 Floral phenology

Floral phenology was studied by tagging the flower buds that would open on the next day and were monitored from an hour before anthesis to until the flower senesced. Anthesis and anther dehiscence was observed using a hand lens (Reddi & Janaki, 1981; Ramasubbu *et al*., 2009). The following major pheno-events were recorded for the plants in the study population.

- Initiation of inflorescences and floral buds.
- Development and the number of flowers per branch/plant.
- The time of flower opening, flower longevity and anther dehiscence.
- Peak period of flowering.
- Initiation, duration and intensity of flowering.
- Duration of flowering.
- Fruit initiation, development, maturation and dehiscence.

4.3 Pollen biology

4.3.1 Pollen production

Pollen production was calculated following the method by Shivanna and Tandon (2014). Mature dehisced anther was placed on a slide marked with parallel gridlines (1 mm apart), in a small drop of water. The anther was squashed gently to release pollen and, covered with a cover slip. Pollen was counted sequentially, covering each parallel line from left to right so that the whole mounted area is covered. Pollen present on the left line was included while counting. The number of pollen produced by a flower was estimated by multiplying the number of pollen produced by an anther with the number of anthers in a flower. The counting was repeated for 10 anthers and the mean with SE was estimated.

4.3.2 Pollen-ovule ratio

Pollen produced per flower was estimated and the average number of ovules per ovary was counted by dissecting young pistils of 25 flowers under a microscope. Pollen-ovule ratio (P/O) was calculated as per the method suggested by Cruden (1977).

$$Pollen - ovule\ ratio = \frac{Mean\ number\ of\ pollen\ grains/flower}{Mean\ number\ of\ ovules/flower}$$

4.3.3 Pollen morphology

4.3.3.1 Surface morphology

Pollen grain collected immediately from flowers after anthesis was transferred through a dehydration series of 30%, 50%, 70% (for storage), 90% and 100% ethyl alcohol. The pollen grains were mounted on stubs and were critical point dried and coated with gold-palladium. The processed pollen grains were

then observed under Zeiss Gemini SEM 300 Microscope (Zeiss, Oberkochen, Germany).

4.3.3.2 Pollen size and shape

Erdtman (1945) categorized six pollen size classes based on the size expressed as length of the longest axis (Table 1).

Table 1. Pollen size classes

Pollen size classes	Length of the longest axis
Very small grains (*sporae perminutae*)	<10 μm
Small grains (*minutae*)	10-25 μm
Medium sized grains (*mediae*)	25-50 μm
Large grains (*magnae*)	50-100 μm
Very large grains (*permagnae*)	100-200 μm
Gigantic grains (*giganteae*)	>200 μm

Erdtman (1952) categorized eight shape classes based on the ratio of polar axis (P) and equatorial axis (E). The ratio between the P and E gives the indication of the shape (Table 2).

Table 2. Pollen shape classes

Shape classes	P/E
Peroblate	<0.50
Oblate	0.50-0.75
Suboblate	0.76-0.88
Oblate-spheroidal	0.89-0.99
Spherical	1.00
Prolate-spheroidal	1.01-1.14
Subprolate	1.15-1.33
Prolate	1.34-2.00
Perprolate	>2.00

4.3.4 Pollen fertility

Pollen fertility percentages were estimated using the acetocarmine staining technique (Shivanna & Tandon, 2014).

Pollen collected from freshly dehisced anthers were placed in a drop of acetocarmine stain taken on a clean microscope slide and were mixed thoroughly. The slide was warmed gently over a spirit lamp and excess stain was removed using blotting paper. After an incubation period of 5 minutes, the slides were observed under Leica DM 2000 digital microscope (Leica Microsystems, Germany) fitted with a Leica DMC 4500 digital camera. Pollen grains with deep, uniform staining were counted as fertile, while unstained or partially stained ones were considered sterile.

4.3.5 Pollen viability

Pollen viability was estimated using the 2,3,5-triphenyltetrazolium chloride (TTC) test (Shivanna & Rangaswamy, 1992). Viable pollen have dehydrogenase group of enzymes that are absent in non-viable pollen. Dehydrogenase enzymes having strong oxidation/ reduction potential, reduces the colourless solution of tetrazolium salt into insoluble red coloured formazone, staining viable pollen red.

- 0.5% tetrazolium chloride solution was prepared in 10% sucrose solution.
- A drop of the solution was taken on a microslide.
- A small amount of pollen was suspended in the TTC drop and distributed uniformly.
- A cover glass was placed carefully over the suspension and the preparation was transferred to a humidity chamber.

- Incubated in dark under room temperature.

After the required incubation period the preparation was observed under a microscope and scored.

4.3.6 Pollen biochemical analysis

Flowers were collected just after anthesis and pollen samples were immersed in a drop of iodine potassium iodide (I_2KI) solution or a drop of Sudan Black or Coomassie Brilliant Blue solution and were examined under a microscope. Dark bluish-black colour (when stained with iodine potassium iodide) indicated the presence of starch, black colour (when stained with Sudan Black) indicated the presence of lipids and dark bluish-black colour (when stained with Coomassie Brilliant Blue) indicated the presence of protein.

Effect of organic and inorganic nutrients on in-vitro pollen germination

In order to estimate the influence of various inorganic nutrients on the percentage of pollen germination in-vitro, the pollen grains were allowed to germinate in various compositions of inorganic solutions as given below.

- 1–30% of sucrose solutions.
- Brewbaker and Kwack medium (Brewbaker & Kwack, 1964).
- Calcium nitrate solutions of 25–500 μg/ml concentration.
- Potassium nitrate solutions of 25–500 μg/ml concentration.
- Boric acid solutions of 25–500 μg/ml concentration.
- Magnesium sulphate solutions of 25–500 μg/ml concentration.

4.4 Stigma biology

4.4. 1 Stigma morphology

Freshly opened flowers were collected and the pistils were observed under a microscope. Stigmas were classified into wet or dry type based on the presence or absence of exudates. Presence or absence of papillae was recorded. The stigma was mounted on a microscopic slide under a cover slip to observe the papillae. Free hand sections of the style were made at the middle of the style and the sections were stained in safranin and were observed under a microscope to observe the nature of the style (Shivanna & Tandon, 2014).

4.4.2 Stigma receptivity

4.4.2.1 Peroxidase activity on stigma surface

Stigma receptivity was estimated by localizing the activity of peroxidases on stigmatic surface (Galen & Plowright, 1987). Hydrogen peroxide, the substrate for the enzyme, results in the release of nascent oxygen bubbles from the stigmatic surface.

Procedure

- Freshly opened un-pollinated flowers were collected.
- Pistils from 6–10 flowers were carefully excised and were kept in humidity chambers. Care was taken not to injure the stigma while handling the flowers.
- A few drops of the reagent solution (1% benzidine prepared in 6% ethanol:H_2O_2:distilled water (4:11:222 v/v) was taken on microscope slides.

- The stigmas were immersed in the solution and the oxygen bubbles released per minute was counted.

- The experiment was repeated 5 times for each time interval and the averages were noted. Floral stages with positive reaction were considered receptive and the stage with greatest mean number of bubbles is the one with maximum receptivity.

4.4.2.2 Cytochemical localization of stigma-surface esterases

Localizing the esterases which are a part of the extracellular matrix of receptive stigmatic surface is a convenient method for estimating stigma receptivity. It involves incubating the stigma in the presence of a substrate, α-naphthyl acetate, which is hydrolyzed by the stigma-surface esteraces to form α-naphthol. α-Naphthol forms a reddish insoluble complex with coupling reagent, fast blue B (Mattsson *et al*., 1974; Ghosh & Shivanna, 1984).

Procedure

- Freshly opened un-pollinated flowers were collected.

- Pistils from 6–10 flowers were carefully excised and were kept in humidity chambers. Care was taken not to injure the stigma while handling the flowers.

- A few drops of solution A and B were taken on separate microslides.

- Stigmas of half the excised pistils were dipped in solution A and the other in solution B.

- After an incubation period of 10–15 minutes the pistils were removed and were washed thoroughly in phosphate buffer.

- The preparations were then observed under Leica DM 2000 digital microscope (Leica Microsystems, Germany) fitted with a Leica DMC 4500 digital camera. The stigmatic surfaces incubated in solutions A and B (Table 3) were compared.

Reaction mixture: The solutions[a] A and B was prepared as given below.

Table 3. Composition of solutions A and B

Reagents	**Solution A (with substrate)**	**Solution B (control, without substrate)**
α-Naphthyl acetate[b]	5 mg	0 mg
Phosphate buffer 0.15M[c]	10 ml	10 ml
Sucrose	1g	1 g
Fast Blue B	25 mg	25 mg

[a] The solution was prepared shortly before use and was used within 15–30 minutes of preparation.

[b] As α-naphthyl acetate is insoluble in phosphate buffer, it was dissolved in a few drops of acetone and then the buffer, sucrose and fast blue B were added and mixed thoroughly.

[c] Preparation of phosphate buffer: Solution X: 0.15 M $NaH_2PO_4.2H_2O$: (20.85g/l of distilled water); Solution Y: 0.15 M $Na_2HPO_4.7H_2O$ (40.24g/l of distilled water) or $Na_2HPO_4.12H_2O$ (53.73g/l of distilled water). Mix 51 ml of solution X with 49 ml of solution Y to get 100 ml of 0.15M phosphate buffer.

4.5 Pollination

4.5.1 Mode of pollination

Time and pattern of anther dehiscence for each species was identified by observing the flowers during anthesis using a hand lens and also by observing the anthers under a microscope. Mode of pollination was identified by studying the role of wind and floral visitors in pollination.

4.5. 2 Role of wind in pollination

To check the possibility of wind pollination vaseline coated slides were hung on the branches of the plants or were affixed on poles amongst the plants.

They were left in the field for 24 hours and were observed under a microscope for pollen deposition.

4.5.3 Floral visitors and their behaviours

Floral visitors were observed and their behaviour was recorded throughout the period of study. Flowers were monitored for visitors from an hour before flower opening up to its closing. The behaviour of insect visitors was documented in field book, photographs and video graphs. The identity, number, visiting time, duration of each bout, time spent on individual flowers on each visit and the frequency of the visits was recorded (Shivanna & Tandon, 2014). The visitors were distinguished as legitimate (those who comes in contact with the anthers and the stigma) or illegitimate (those insects that rob pollen without touching the anthers and/or stigma).

Specimens of insect visitors were collected using nets or plastic containers and were conditioned using ethyl acetate. The specimens were identified with the help of experienced entomologists.

4.5.4 Pollination efficiency

Stigmas were observed using a head mount or handheld magnifier after the flowers closed in field conditions to evaluate the presence of pollen. To study the pollination efficiency by individual visitors, stigmas were observed immediately after the first visit of a particular insect on virgin stigmas.

4.6 Breeding system

Studies of breeding system were performed at the two selected natural population for individual species (Kaul & Koul, 2009).

4.6.1 Natural/Open pollination (OP)

Flowers were tagged and allowed to be pollinated under natural conditions. Their fruit set and seed set values were recorded.

4.6.2 Autogamy (spontaneous selfing)

Flowers were tagged and bagged using butter paper bags and were left undisturbed to check for the occurrence of spontaneous selfing or autogamous pollination.

4.6.3 Manual self-pollination (geitonogamy)

Flowers were emasculated an hour before anthesis and were pollinated using pollen from other flowers of the same plant. After pollinating the flowers were bagged and left to set fruit.

4.6.4 Manual cross-pollination (xenogamy)

Flowers were emasculated an hour before anthesis and were pollinated using pollen from flowers of a different population. After pollinating the flowers were bagged and left to set fruit.

4.6.5 Apomixis

Flowers were emasculated an hour before anthesis and were bagged using butter paper bags to test for non-pseudogamous apomixes.

4.7 Fruit and seed biology

4.7.1 Fruit and seed set

Flowers from each of the treatments for breeding system were monitored from the day of pollination to maturity. Mature fruits were counted to evaluate the fruiting percentages. The type and pattern of fruit dehiscence was observed. To estimate the percentage of fruit set inflorescences were tagged at

random. Number of seeds per fruit was recorded for all the treatments for breeding system. Percentages of fruit and seed sets were calculated by the following formula:

$$Fruit\ set\ (\%) = \frac{Number\ of\ fruits\ per\ infructescence}{Number\ of\ flowers\ per\ inflorescence} \times 100$$

$$Seed\ set\ (\%) = \frac{Number\ of\ seeds\ per\ fruit}{Number\ of\ ovules\ per\ pistil} \times 100$$

4.7.2 Flower-fruit ratio and ovule-seed ratio

The total number of flowers formed in an inflorescence was observed along with the number of fruits developed in the inflorescence. The ratio between the two was found and recorded as the flower to fruit ratio. The ratio of the total number of ovules in an inflorescence against the number of seeds formed from that inflorescence gave the ovule to seed ratio.

4.7.3 Fruit and seed dispersal mechanism

Regular field visits were conducted to observe and record the agents of fruit or seed dispersal.

4.7.4 Seed germination

Seed germination tests were conducted as a test for seed viability. Seeds were soaked in water for 30 minutes and were placed in petridishes lined with cotton. The germination patterns of the seeds of each species was closely monitored and recorded.

Various methods such as acid treatments, stratification (chilling at 0–10^{0}C) were adapted to break dormancy of *D. montanum* and *F. scandens* seeds.

Seed germination was also observed in the field and seeds were also kept in soil in pots in the garden to germinate.

4.8 Statistical analysis

All the data collected during the study was analyzed using Microsoft Excel 2010. One way ANOVA was conducted with 0.05% level of significance to estimate the significance of variation in results under the different sets of conditions.

5. RESULTS

5.1 Commelina diffusa Burm.f.

Commelina diffusa is a widespread weed, usually found in damp shady places near water and also in open swamps, marshes and sewers (Fig. 2 A; Fig. 3 A & B). This species is also found as a weed in cultivated fields (Khanna & Saran, 2001).

Extensively spreading, decumbent annual herbs lacking a definite base; roots profusely from the nodes. Stems terete, green, puberulous; internodes 3–6 cm long. Leaf sheath 1.5 cm long, margins ciliate. Lamina 4–6.5 × 1.5–2.5 cm, ovate-lanceolate, acute-acuminate at apex, upper surface glabrous or puberulous, lower surface glabrous. Flowers born in two cincinni enclosed within a spathe; spathes leaf-opposed, margins free; upper cincinnus exerted on a 1.3 cm long peduncle while the lower cincinnus on a 0.6 cm long peduncle. The upper cincinnus bears only one (very rarely 2) male (rarely 1 bisexual) flower with a 0.5 cm long pedicel and the lower bears 2–3 (rarely 4) bisexual flowers with pedicels about 0.2 cm long. Flowers trimerous. Sepals 3, the medial one is boat shaped while the lateral ones are fused by half their length. Petals 3, blue, the two lateral deltoid ones occupy a posterior position whereas the smaller medial one is anterior. Three stamens with dimorphic anthers: two lateral stamens with purple, basifixed, bithecous anthers and a medial stamen with yellow shield shaped anther. The medial stamen is positioned in between the lateral petals, facing downwards and the lateral stamens flank the lateral petals. Filaments of the lateral stamens in male flowers are about 7 mm long and that of the medial stamen is 6.5 mm where as in the bisexual flowers both the filaments are about 6 mm. In the bisexual flowers the medial stamen is oriented directly opposite to and facing the stigma. There are two (rarely 3) staminodes (5 mm long in the male and 6 mm

in the bisexual flowers) with 4 lobed yellow antherodes. Style 6 mm long with a capitate stigma. Capsules 4–5 seeded (Fig. 4 A-L).

5.1.1 Phenology

5.1.1.1 Population phenology

Commelina diffusa is an annual and it propagates through both vegetative and sexual means. With the arrival of first rain, the seeds germinate, and the plants spread over a given area. It thrives in areas with continuous supply of water, especially alongside the sewers. Plants initiate flowering after about a month of vegetative growth and dies after about three to four months of profuse flowering.

Flowering was observed from June to December in natural populations, August to October being the peak period. Flowering period lasts for about 3 months within a population.

5.1.1.2 Flowering phenology

The spathe starts flowering after 8–12 days of initiation (Fig. 5. A-H). The upper cincinnus bears only one (very rarely 2) male (rarely 1 bisexual) flower and the lower bears 2–3 (rarely 4) bisexual flowers. Within a spathe the male flower on the upper cincinnus and the first bisexual flower on the lower cincinnus flowers the same day in majority of the spathes. The second flower on the lower cincinnus blooms the next day and the third and fourth opens after a gap of 1 to 4 days. Occasionally the bisexual flower on the lower cincinnus flowers first and the male flower on the upper cincinnus opens only the next day.

5.1.1.3 Intra-floral phenology

Anthesis takes place around 07.00 to 07.30 am. The flowers remain open for 4–5 hours. In 92% of the inflorescence studied the male flower in a spathe opened about 5–6 minutes earlier than the bisexual ones. Rarely the male

flower opened a day after the first bisexual flower in the same spathe (Fig. 6 A-H). Anther dehiscence occurred within 5 minutes of anthesis (Table 17). Towards the end of anthesis, as petals began to wither, the style began coiling and got closer to the medial stamen (Fig. 6 G & H). The petals deliquesced and the flowers withered completely by the afternoon.

5.1.2 Sex expression of flowers in the spathes

The sexuality of the flowers of the lower cincinnus (third order) was observed to be influenced by the fruit set in the first and second order flowers. When the first and second order flowers set fruit in the lower cincinnus, the third order flower formed was generally a male flower but if either or both of the first and second order flowers failed to set fruit the third order flower was more likely to be bisexual (Fig. 7 C-E). More details on sex expression and the composition of flowers in the spathes of *C. diffusa* are given in the table 4.

Table 4. *Commelina diffusa*: Sex expression and composition of flowers in the spathes

No. of flowers per spathe	Frequency of such spathes	No. and sex expression of flowers		Position of flowers that set fruit
		Upper cincinnus	Lower cincinnus	
3	39	1M	2Bi	1st and 2nd
4	33	1M	2Bi+1M	1st and 2nd
4	16	1M	3Bi	2nd
5	9	1M	3Bi+R	2nd and 3rd and rarely 1st
3	2	1Bi	2Bi	1st or all of the lower cincinnus
4	1	1Bi	1Bi+M	1st of the lower cincinnus

M, male; Bi, bisexual; R, rudimentary

5.1.3 Pollen biology

5.1.3.1 Pollen morphology

Pollen grains are prolate, spinulose and monolete. Pollen produced by both the lateral and medial stamen of the bisexual flowers was significantly larger when compared to those of the male flowers (Fig. 8 A & B). Pollen produced by the lateral stamen of the male flowers was 41.70±0.20 μm (P) and 26.42±0.15 μm (E) and that of the lateral stamen of the bisexual flowers was 51.36±0.38 μm (P) and 33.96±0.26 μm (E). Pollen produced by the medial stamen of the male flowers was 42.10±0.28 μm (P) and 27.93±0.27 μm (E) and those of the medial stamen of the bisexual flower was 52.44±0.43μm (P) and 34.25±0.31μm (E) (n=50).

5.1.3.2 Pollen biochemical analysis

The pollen grains stained with I_2KI solution became brownish black, indicating the presence of starch and those stained with Sudan Black became black indicating the presence of lipid. On staining with Coomassie Brilliant Blue the pollen grains became blue tinged indicating the presence of protein (Fig. 8 F-H).

5.1.3.3 Pollen production

The medial stamen produced significantly more pollen than the lateral ones ($p<0.05$) in both the male and bisexual flowers. Mean number of pollen produced by the lateral stamen (L) of the male and bisexual flowers were calculated to be 2920±347and 2260±159 and that by the medial stamen (M) in male and bisexual flowers were 4320±239 and 3020±234 respectively (Table 5).

5.1.3.4 Pollen-ovule ratio

Average pollen production per flower was estimated for flowers of the different orders and is given in the table 5.

Table 5. *Commelina diffusa*: Average pollen production per flower and the pollen-ovule ratios

Flower order	Pollen count per lateral stamen	Pollen count per medial stamen	Pollen count per flower	No. of ovules per flower	Pollen-ovule ratio
Male	2920	4320	10160	0	-
Bisexual 1	2260	3020	7540	5	1508
Bisexual 2	1600	2126	5326	5	1065
Bisexual 3	928	1110	2966	5	593

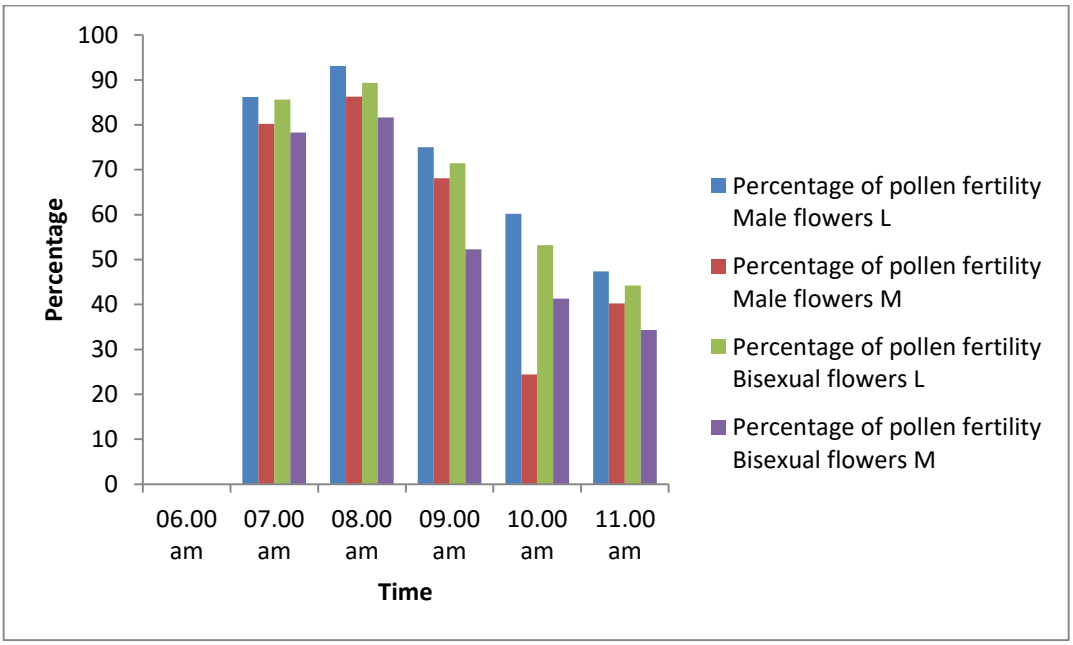
5.1.3.5 Pollen fertility and sterility

Acetocarmine staining technique showed (Fig. 8 D) that the pollen from the lateral stamen was significantly more fertile than that from the medial stamen in both male and bisexual flowers ($p<0.05$). Maximum fertility was observed around 07.00–09.00 am in all the cases (Table 6; graph 1).

Table 6. *Commelina diffusa*: Pollen fertility - acetocarmine test

Sl No.	Time of observation	Percentage of pollen fertility±S.E			
		Male flowers		Bisexual flowers	
		L	M	L	M
1	06.00 am	-	-	-	-
2	07.00 am	86.24±0.12	80.23±0.41	85.6±0.22	78.31±0.22
3	08.00 am	93.15±0.13	86.27±0.25	89.36±0.21	81.62±0.52
4	09.00 am	75.04±0.28	68.12±0.32	71.42±0.42	52.26±0.22
5	10.00 am	60.21±0.24	24.42±0.34	53.24±0.31	41.32±0.43
6	11.00 am	47.36±0.17	40.26±0.92	44.26±0.32	34.35±0.26

Graph 1. *Commelina diffusa*: Pollen fertility - acetocarmine test



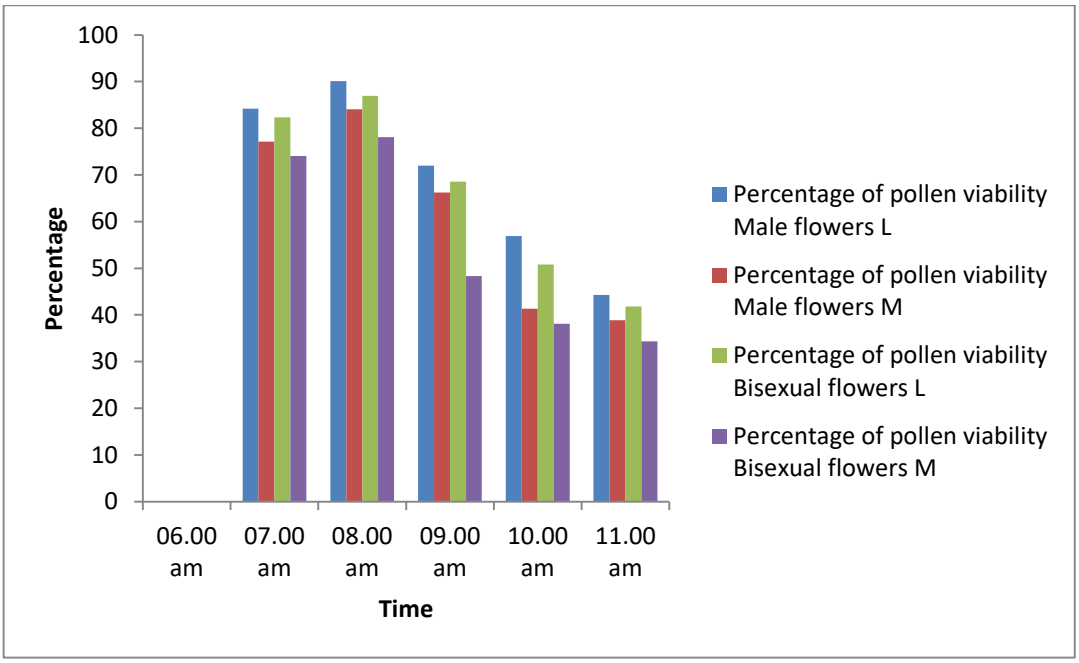
5.1.3.6 Pollen viability

Pollen from the lateral stamen was significantly more viable than that from the medial stamen in both male and bisexual flowers and both lateral and medial stamen of the male flowers produces significantly more viable pollen than the corresponding stamens of the bisexual flowers ($p<0.05$). Maximum viability was observed around 07.00–09.00 am and then steadily decreased in all the cases (Fig. 8 C; table 7; graph 2).

Table 7. *Commelina diffusa*: Pollen viability - tetrazolium test

Sl. No.	Time of observation	Percentage of pollen viability±S.E			
		Male flowers		Bisexual flowers	
		L	M	L	M
1	06.00 am	-	-	-	-
2	07.00 am	84.20±0.62	77.13±0.20	82.36±0.12	74.03±0.42
3	08.00 am	90.09±0.35	84.09±0.45	86.98±0.17	78.12±0.45
4	09.00 am	72.02±0.52	66.22±0.32	68.54±0.32	48.36±0.23
5	10.00 am	56.91±0.31	41.32±0.24	50.77±0.31	38.12±0.33
6	11.00 am	44.26±0.27	38.86±0.91	41.82±0.60	34.36±0.42

Graph 2. *Commelina diffusa*: Pollen viability - tetrazolium test



5.1.3.7 Effect of organic and inorganic nutrients on in-vitro pollen germination

In-vitro pollen germination studies (Fig. 8 E) showed that maximum germination percentage (56.05±0.27 and 48.60±0.43 for male and bisexual flowers respectively) and maximum pollen tube length (128.82±0.27 and 130.06±1.82 for male and bisexual flowers respectively) was observed in Brewbaker & Kwack's medium. Sucrose solution of 10% gave the next best result with 11.18±0.38% germination and 89.33±0.93 µm pollen tube length in male flowers and 9.52±0.46% germination and 79.90±0.16 µm mean pollen tube length in bisexual flowers (Table 8; graph 3 & 4).

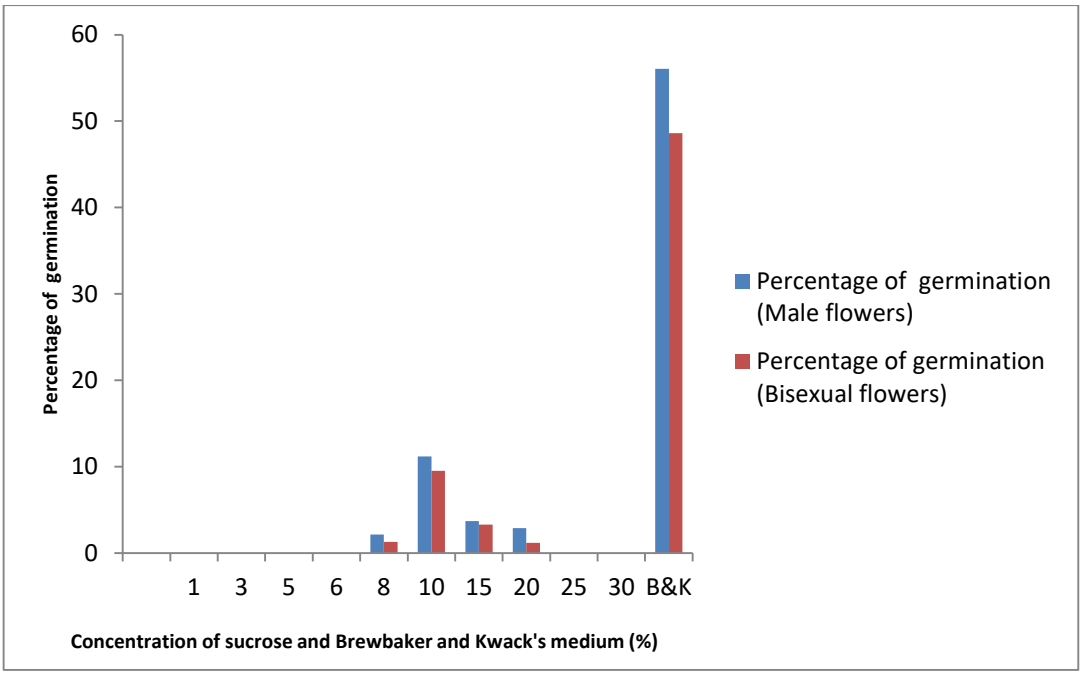
Maximum percentage of germination (17.16±1.57%) was observed in calcium nitrate solution (Table 9, graph 5 & 6) of 200 µg/ml while maximum pollen tube length was obtained in 300 µg/ml (17.16±1.57 µm). 100 µg/ml solution of potassium nitrate (Table 10; graph 7 & 8) gave the maximum percentage of pollen germination (10±2%) as well as the maximum pollen tube length

(48.36±4.2 µm). Pollen germination was observed in boric acid solution (Table 11; graph 9 & 10) of 100 µg/ml with an average of 12.32±1% germination and 62.09±2.2 µm pollen tube length and in 200 µg/ml of magnesium sulphate solution (Table 12; graph 11 & 12) with average germination of 4.62±3.6% and pollen tube length of 32.08±8.2 µm.

Table 8. *Commelina diffusa*: In-vitro pollen germination in sucrose and Brewbaker & Kwack's medium.

Concentration (%)	**Male flowers**		**Bisexual flowers**	
Sucrose	**Pollen germination ± S.E. (%)**	**Pollen tube length ± S.E. (µm)**	**Pollen germination ± S.E. (%)**	**Pollen tube length ± S.E. (µm)**
1	-	-	-	-
3	-	-	-	-
5	-	-	-	-
8	2.16±0.92	56.78±0.12	1.3±0.33	62.66±0.92
10	11.18±0.38	89.33±0.93	9.52±0.46	79.93±0.16
15	3.7±0.85	44.36±0.62	3.3±0.96	72.68±0.98
20	2.9±0.30	52.22±1.2	1.2±0.22	69.01±0.36
25	-	-	-	-
30	-	-	-	-
Brewbaker & Kwack's medium	56.05±0.27	128.82±0.36	48.60±0.43	130.06±1.82

Graph 3. *Commelina diffusa*: Effect of sucrose and Brewbaker and Kwack's medium - pollen germination



Graph 4. *Commelina diffusa*: Effect of sucrose and Brewbaker and Kwack's medium - pollen tube length

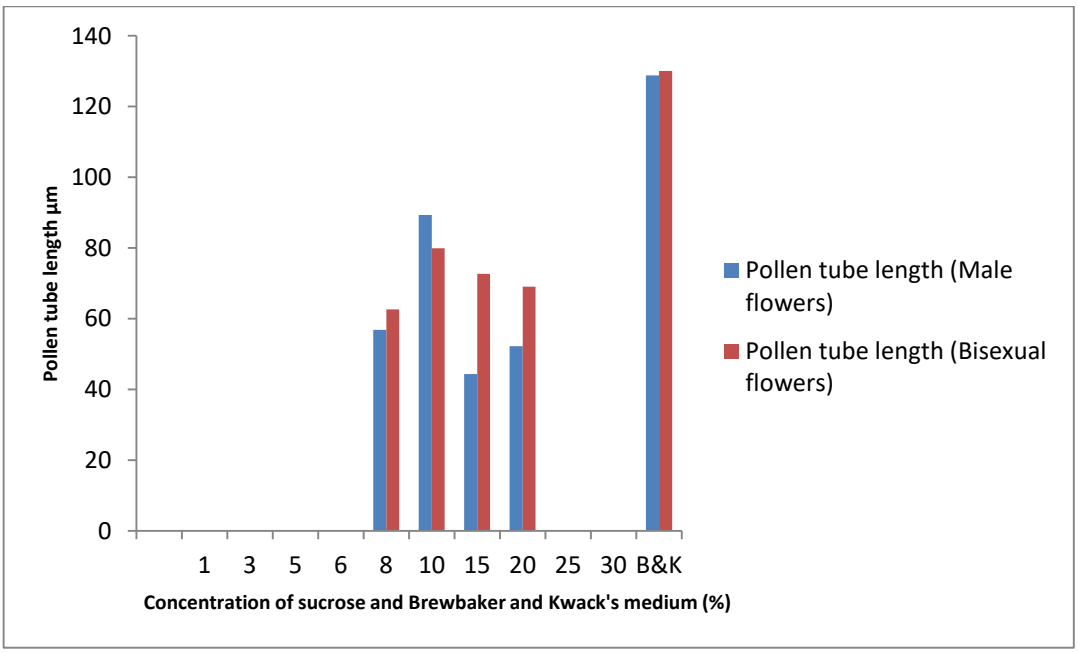
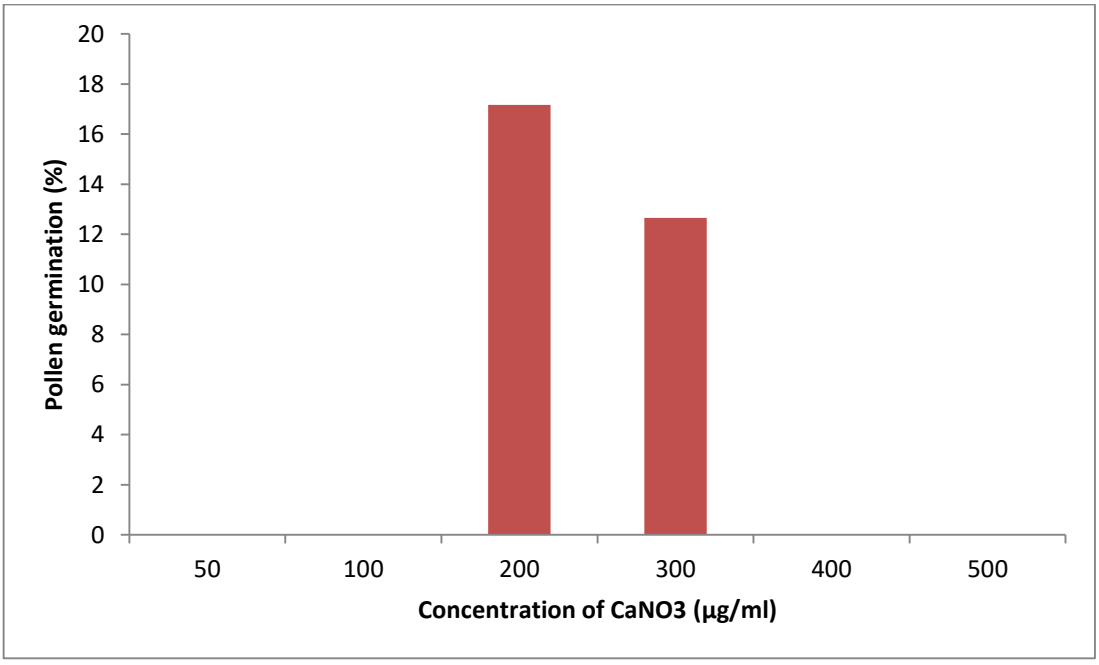


Table 9. *Commelina diffusa*: In-vitropollen germination - effect of calcium nitrate

Sl. No.	Concentration of $CaNO_3$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	50	-	
2	100	-	
3	200	17.16±1.57	49.42±5.30
4	300	12.65±0.84	17.16±1.57
5	400	-	-
6	500	-	-

Graph 5. *Commelina diffusa*: Effect of calcium nitrate - pollen germination



Graph 6. *Commelina diffusa*: Effect of calcium nitrate - pollen tube length

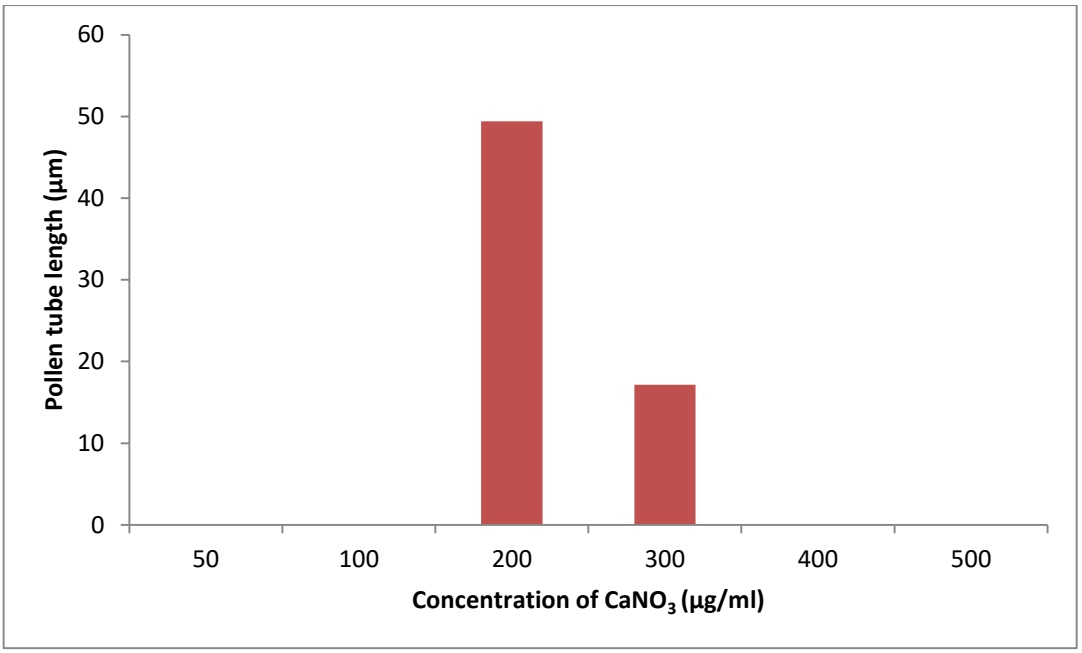
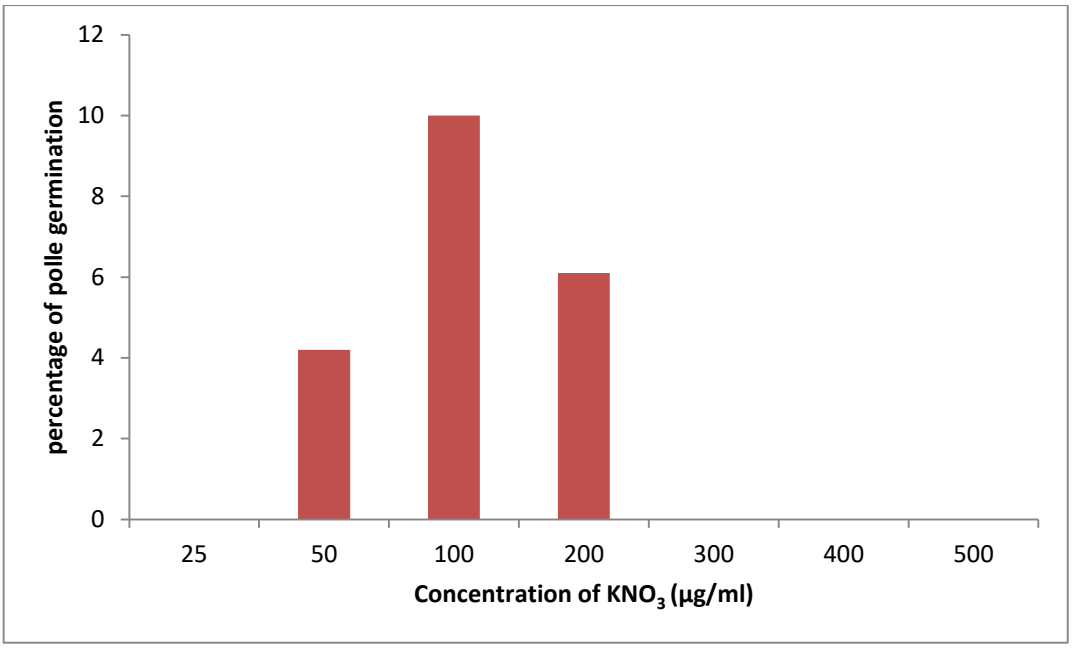


Table 10. *Commelina diffusa*: In-vitro pollen germination - effect of potassium nitrate

Sl. No.	Concentration of KNO_3 (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	4.2 ±1.1	42.16±3.4
3	100	10±2	48.36±4.2
4	200	6.1±2.6	42.22±4.8
5	300	-	-
6	400	-	-
7	500	-	-

Graph 7. *Commelina diffusa*: Effect of potassium nitrate - pollen germination



Graph 8. *Commelina diffusa*: Effect of potassium nitrate - pollen tube length

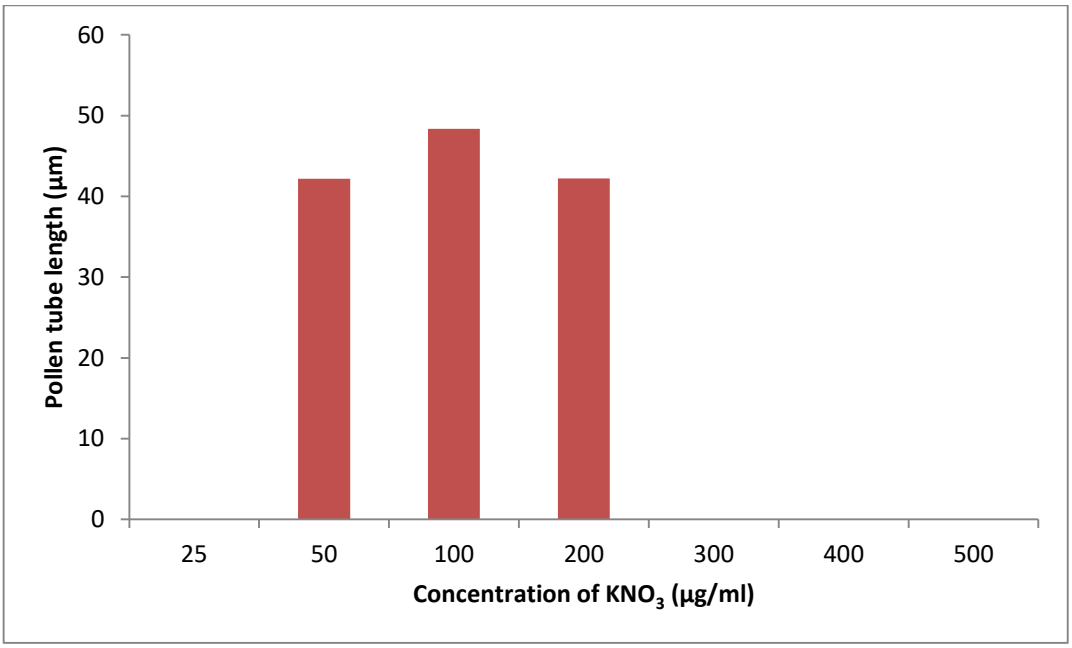
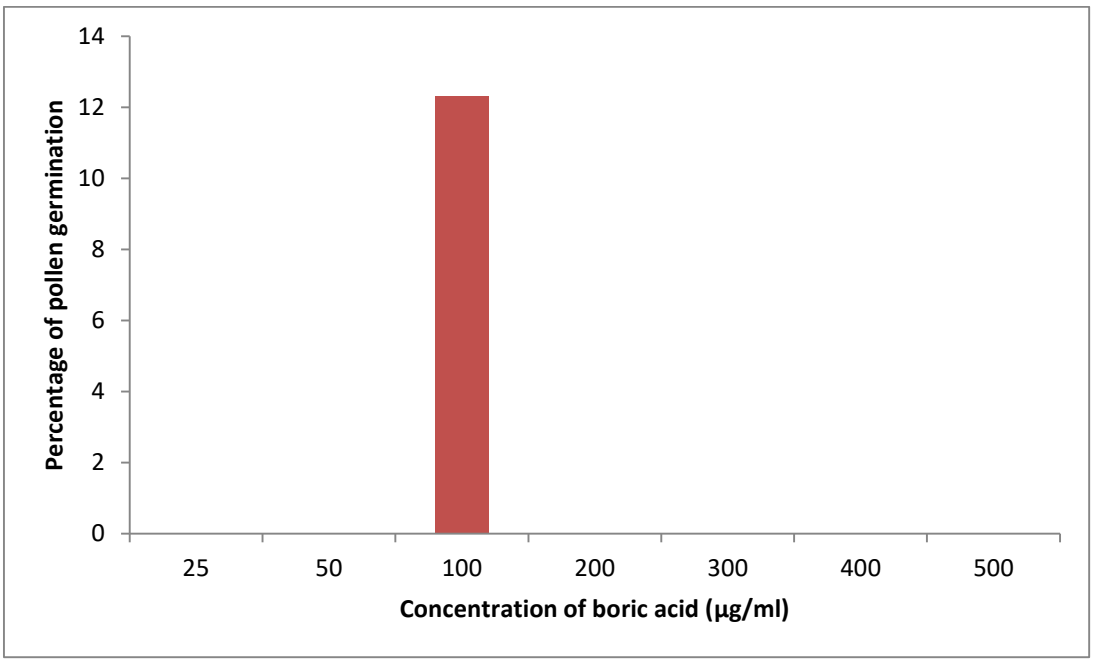


Table 11. *Commelina diffusa*: In-vitro pollen germination - effect of boric acid

Sl. No.	Concentration of boric acid (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	12.32±1	62.09±2.2
4	200	-	-
5	300	-	-
6	400	-	-
7	500	-	-

Graph 9. *Commelina diffusa*: Effect of boric acid - pollen germination



Graph 10. *Commelina diffusa*: Effect of boric acid - pollen tube length

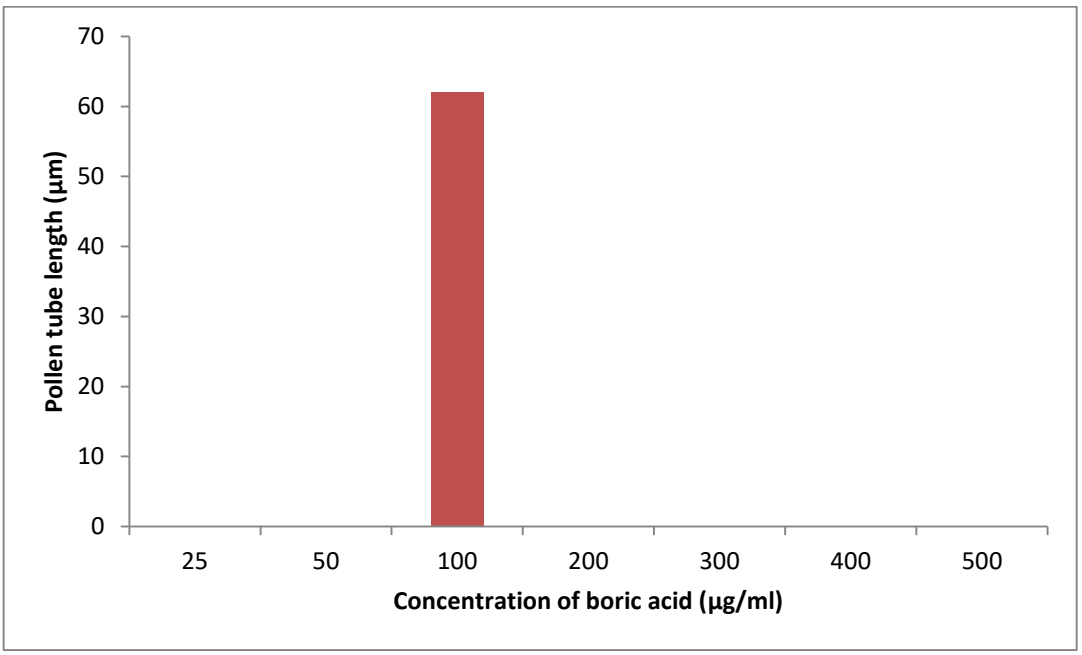
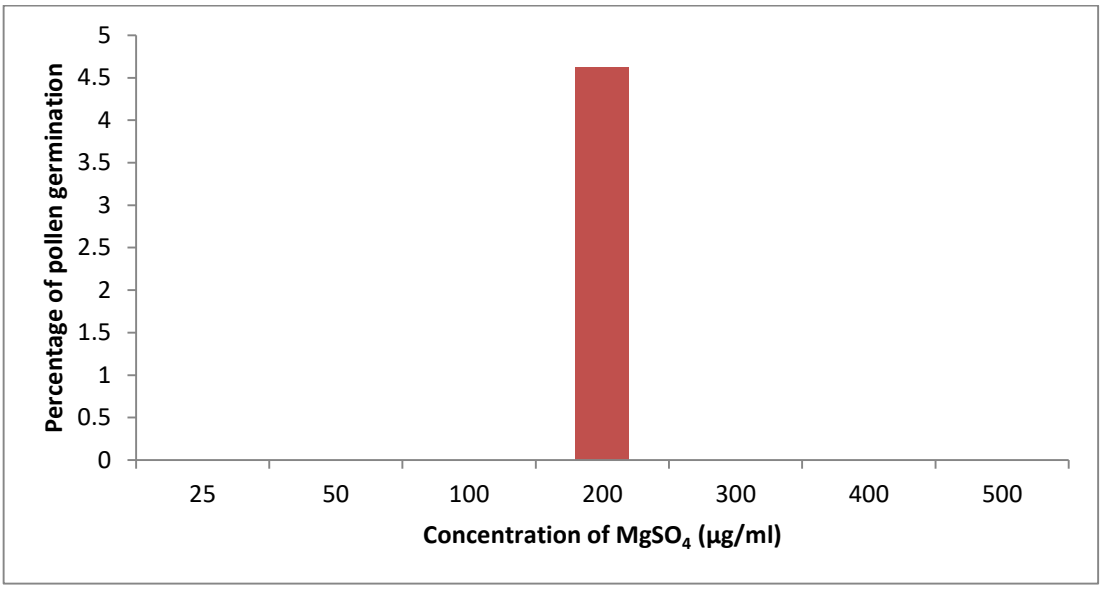


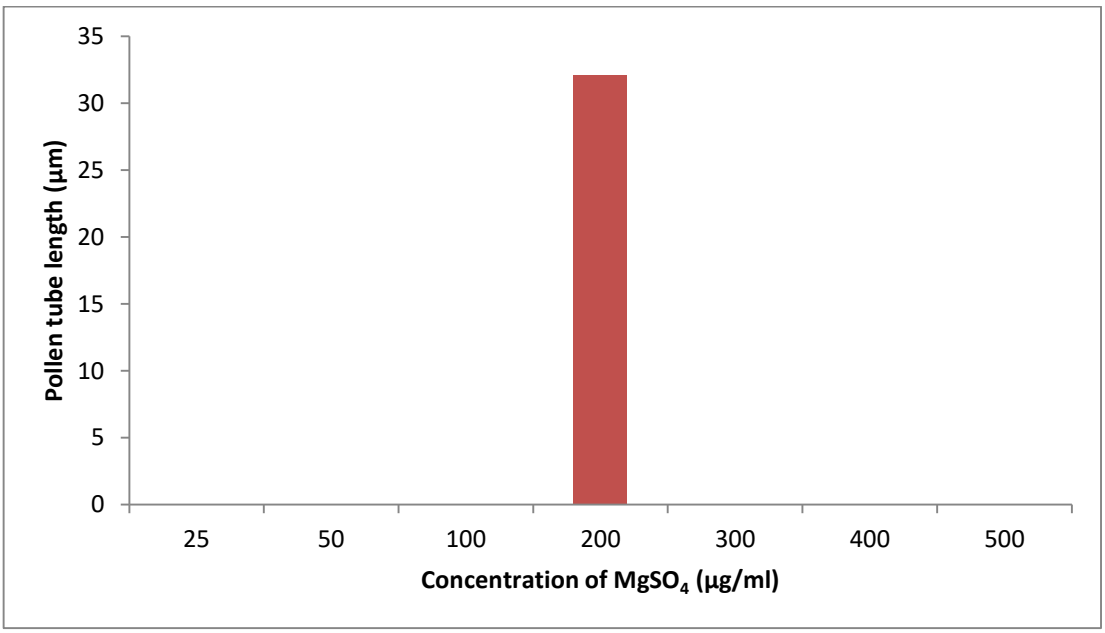
Table 12. *Commelina diffusa*: In-vitro pollen germination - effect of magnesium sulphate

Sl. No.	Concentration of $MgSO_4$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	-	-
4	200	4.62±3.6	32.08±8.2
5	300	-	-
6	400	-	-
7	500	-	-

Graph 11. *Commelina diffusa*: Effect of magnesium sulphate - pollen germination



Graph 12. *Commelina diffusa*: Effect of magnesium sulphate - pollen tube length



5.1.4 Stigma biology

5.1.4.1 Stigma morphology

The stigma is of dry type and the stigmatic surface measures about 150.49±0.36 μm and the entire surface is covered by papillae of about 99.89±1.53 μm. The style is solid.

5.1.4.2 Stigma receptivity

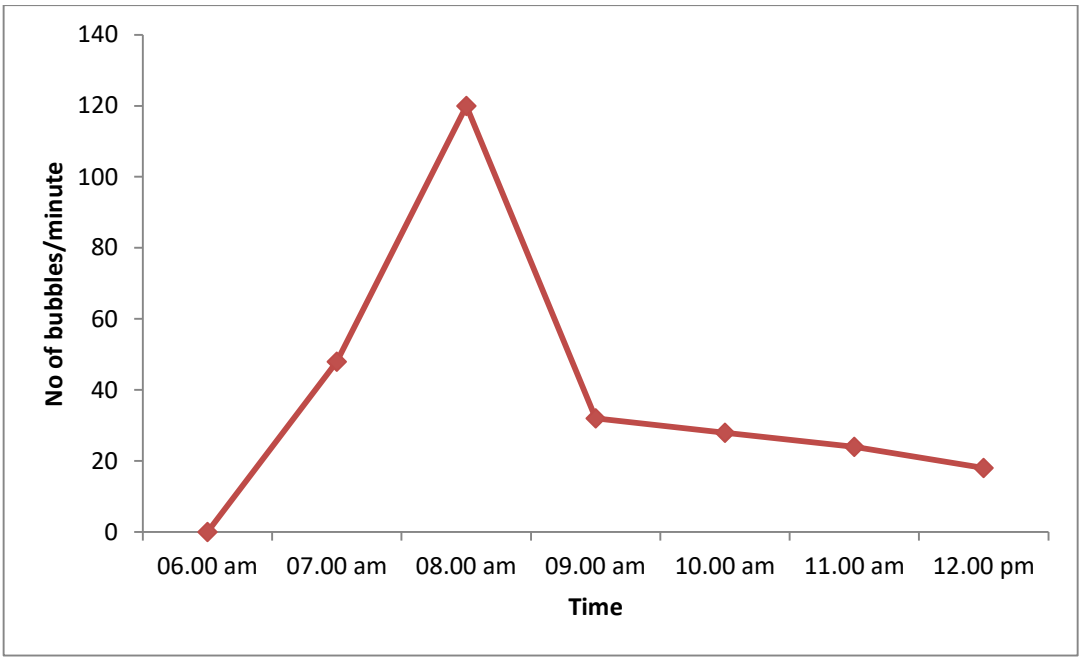
5.1.4.2.1 Stigma receptivity- hydrogen peroxide test

Stigmatic surface showed maximum receptivity around 08.00 am in hydrogen peroxide test (Fig. 9 C & D; table13; graph 13).

Table 13. *Commelina diffusa*: Stigma receptivity - hydrogen peroxide test

Sl. No	Time of observation	No. of bubbles/minute ± S.E.
1	06.00 am	-
2	07.00 am	48±2
3	08.00 am	120±4
4	09.00 am	32±3
5	10.00 am	28±2
6	11.00 am	24±1
7	12.00 pm	18±2

Graph 13. *Commelina diffusa*: Stigma receptivity - hydrogen peroxide test



5.1.4.2.2 Cytochemical localization of stigma-surface esterases

Cytochemical localization of stigma-surface esterases using α-naphthyl acetate demonstrated that the stigmatic surface was most receptive during a period between 07.00–08.00 am (Fig. 9 A & B).

5.1.5 Pollination

5.1.5.1 Mode of pollination

Mode of pollination is entomophilous and autogamous.

5.1.5.1.1 Role of wind in pollination

Examination of the microscopic slides under a microscope revealed an absence of pollen grains, thus ruling out wind pollination for the species.

5.1.5.1.2 Floral visitors and their behaviours

On most days insect visits started along with anthesis. Insects, especially those of the *Halictu*s spp. (Halictidae) would force into the barely open flowers and collect pollen.

Four species of *Halictus*, two species of *Ceratina* (Apidae), *Apis dorsata* (Apidae), *Amegilla zonata* (Apidae), *Tetragonula iridipennis* (Apidae), an unidentified species of the family Syrphidae, *Blatella* sp. (Ectobiidae) and *Lilioceris merdigera* (Chrysomelidae) were observed visiting the flowers (Fig. 10–18). Of these, *L. merdigera* is a beetle that eats away the floral parts and destroys the flowers and *Blatella* sp. is a pollen robber, which sometimes facilitates pollination. All the rest were identified as efficient pollinators. *Halictus* sp. 4 seems to be the most efficient with respect to its high frequency of visits and the time it spends foraging on the flowers.

Insects on their initial visits, goes directly to the medial stamen and collect pollen from them and then either move on to the next flower or sometimes move to the lateral stamens. Insect approach to the medial or lateral stamens seems random when the visits are repeated. At the beginning of the foraging hour right after anthesis, insects went to the male flower in a spathe before visiting the bisexual ones and the visits became random.

Table 14. *Commelina diffusa*: Floral visitors and their behaviours

	Name of the taxa with family	Nature	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality	
							Field 1	CU campus
1	*Halictus* sp. 1 Halictidae	Pollinator	07.00 am-11.30 pm	30-40 seconds	+++	High	✓	-
2	*Halictus* sp. 2 Halictidae	Pollinator	07.30 am-11.30 pm	20-30 seconds	++	high	✓	-
3	*Halictus* sp. 3 Halictidae	Pollinator	07.00 am-11.30 pm	20-30seconds	+++	High	✓	✓
4	*Halictus* sp. 4 Halictidae	Pollinator	07.00 am-11.30 pm	30-40seconds	+++	High	-	✓
5	*Api dorsata* Apidae	Pollinator	07.00 am-11.30 pm	25-35 seconds	+++	high	✓	✓
6	*Ceratina* sp. 1 Apidae	Pollinator	07.45 am-11.30 pm	20-35 seconds	++	intermediate	✓	-
7	*Ceratina* sp. 2 Apidae	Pollinator	07.30 am-11.30 pm	20-35seconds	++	intermediate	✓	✓
8	*Amegilla zonata* Apidae	Pollinator	08.30 am-11.00 pm	3-10 seconds	+++	high	✓	✓
9	*Tetragonula iridipennis* Apidae	Pollinator	08.00 pm-11.00 pm	10-20 seconds	++	intermediate	✓	✓
10	Unidentified sp. 1 Syrphidae	Pollinator	08.30 am -11.00 pm	20-30 seconds	++	Low	✓	✓
11	*Blatella* sp. Ectobiidae	Pollinator/Pollen robber	10.30 am 11.00 am	30-60 seconds	+	low		✓
12	*Lilioceris merdigera* Chrysomelidae	predator	Random	-	-	-	✓	✓

Stigma touch: +++ very good; ++ good; + poor

Frequency of visits: High (5–30 visits/day); Intermediate (1–5 visits/day); Low (<1 visit/day).

Halictus sp. and *Amegilla zonata* will hover for a while before landing on a flower and after visiting all the flowers in a patch once or twice they usually left the patch for a while. *A. dorsata*, usually aligns itself in front of the flower and hover before landing. *Ceratina* spp. and *T. iridipennis* land on the stamens and move around within the flower, while working furiously at the stamens. They sometimes move over to the petals to navigate within the flower. *Blatella* sp. works on the stamens, eating pollen grains and sometimes as they move on the flower, they come in contact with the stigma, but the pollen grains dusted on them are very low when compared to the other insects. More details on the floral visitors are given in the table 14.

5.1.5.2 Pollination efficiency

Stigmas were observed under a microscope after the flowers closed. Stigmas of 78% of flowers showed the presence of pollen.

To study the pollination efficiency by individual visitors, stigmas were observed right after the first visit. *Halictus* sp. 1 (Fig. 10) was found as the most efficient pollinator of *C. diffusa.*

5.1.6 Breeding system

The fruit set and seed set percentages from each of the pollination treatments are shown in the tables 15 & 16 and graphs 14 & 15.

5.1.6.1 Apomixis

No fruit set was observed in flowers bagged after emasculation, prior to anthesis.

5.1.6.2 Autogamy

Fruit set in flowers bagged before anthesis was 71.43% and 73.33% at population 1 and population 2 respectively.

5.1.6.3 Manual self-pollination (MSP)

Pollinating flowers by pollen from the same flowers showed 88.57% of fruit set in population 1 and 86.67% in population 2.

5.1.6.4 Manual cross-pollination (MCP)

Flowers pollinated by pollen from other plants showed 85.71% of fruit set in population 1 and 83.33% of fruit set in population 2.

5.1.6.5 Open pollination (OP)

Flowers left to be pollinated by natural pollinators under natural conditions showed 54.29% of fruit set in population 1 and 43.33% in population 2.

Table 15. *Commelina diffusa* Breeding system, Population 1

Sl. No.	Treatments	POP 1- Vellimadukunnu					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seeds
1	Apomixis	35	0	0	0	5	0
2	Autogamy	35	25	71.43	112	5	64.00
3	MSP	35	31	88.57	131	5	74.86
4	MCP	35	30	85.71	132	5	75.43
5	OP	35	19	54.29	90	5	51.43

Graph 14. *Commelina diffusa*: Breeding system - fruit set and seed set at population 1

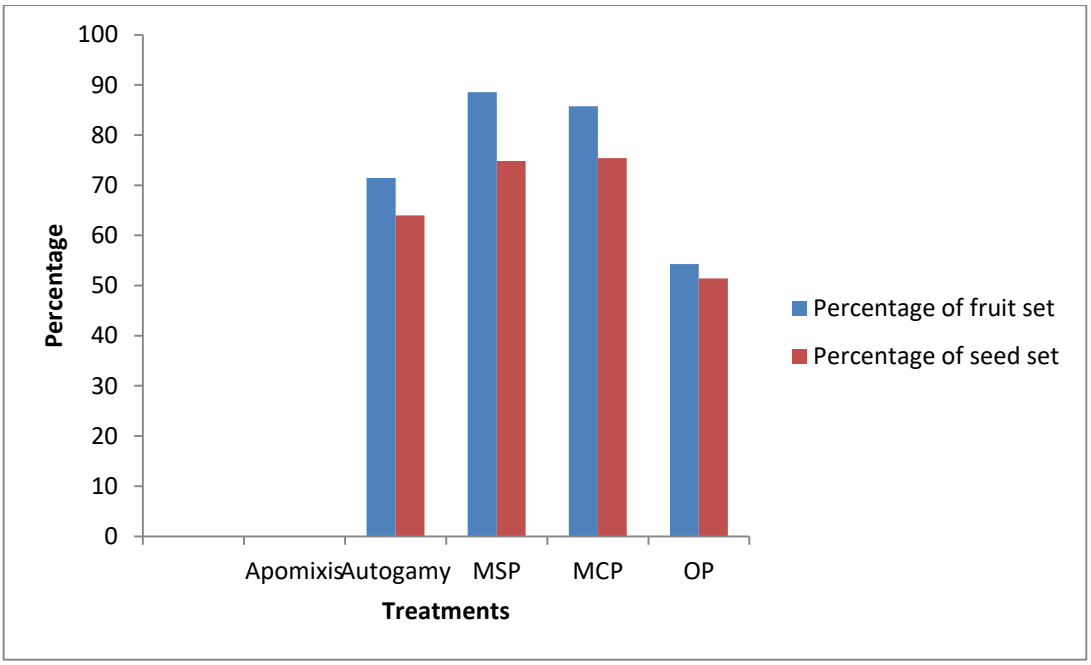
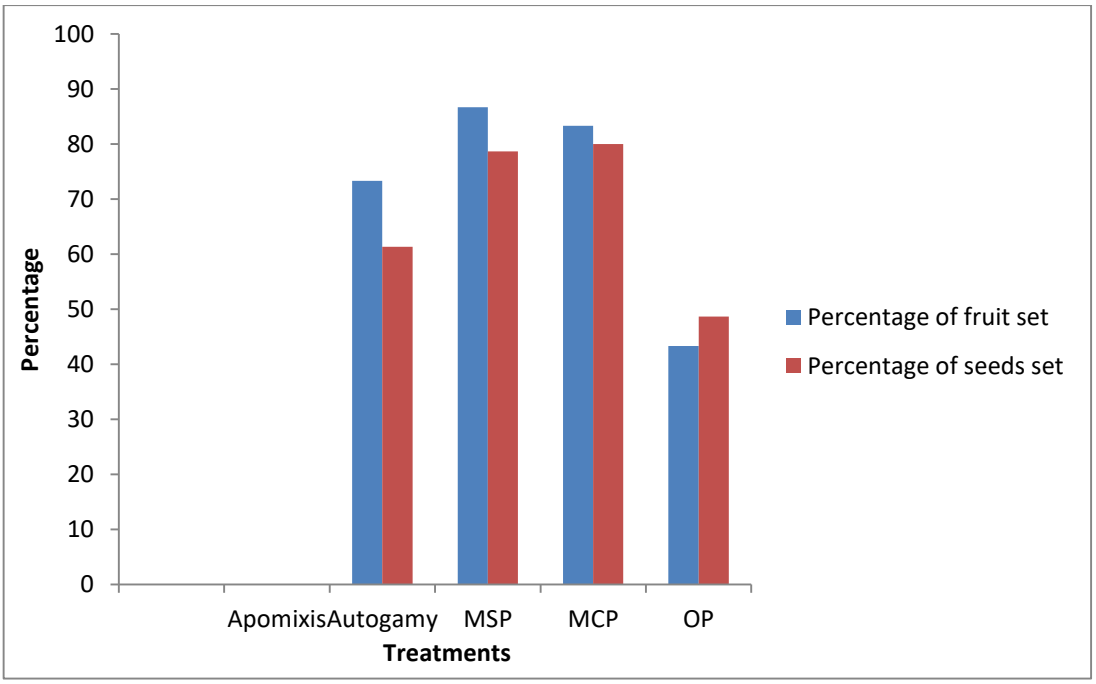


Table 16. *Commelina diffusa*: Breeding system, Population 2

Sl. No.	Treatments	POP 2- CU campus					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seeds
1	Apomixis	30	0	0	0	5	0
2	Autogamy	30	18	73.33	92	5	61.33
3	MSP	30	26	86.67	118	5	78.67
4	MCP	30	25	83.33	153	5	80.00
5	OP	30	13	43.33	73	5	48.67

Graph 15. *Commelina diffusa*: Breeding system - fruit set and seed set at population 2



5.1.7 Fruit and seed biology

After fertilization, the petals, sepals and other floral parts slowly perishe and the fruit remains enclosed within the spathe.

The capsules are oblong, glabrous, ellipsoid to globose, trilocular (Fig. 4 K), 2-valved, brown, 6–9 mm long, the ventral locules 2-seeded, dehiscent, the dorsal locule 1-seeded, indehiscent. Seeds light brown to black, elliptic in outline, *ca*. 3 mm long, dorsiventral, ventral surface planar, dorsal surface convex. Hilum linear on the ventral surface. Seed surface doubly reticulate, and ribbed. Primary cells polygonate with depressed faces and elevated edges and secondary cells also polygonate with depressed face and elevated ridges, giving a network like appearance.

5.1.7.1 Flower-fruit ratio and ovule-seed ratio

Under natural conditions, the flower to fruit set ratio was 65:32 and the ovule to seed set ratio was 325:163.

5.1.7.2 Seed germination

Mature seeds kept in a petri dish over cotton soaked in water showed 6% germination whereas seeds sowed in pots under nursery condition showed 24% germination.

Commelina diffusa shows hypogeal and remote tubular germination where a non haustorial part of the cotyledonary hyperphyll (apocole) grows out of the seed and creates some distance between the seed and the cotyledonary sheath. In this species, the apocole is very short and is attached with the cotyledonary sheath at the medial region (Fig. 19 A-H).

Table 17. *Commelina diffusa*: Summary of floral characters

Sl. No.	Floral characters	Observations			
1	Flowering period	June to December			
2	Flower Type	Zygomorphic, andromonoecious			
3	Flower colour	Blue			
4	Odour	Absent			
5	Nectar	Absent			
6	Anthesis time	07.00-07.30 am			
7	Anther dehiscence time	07.00-07.35 am			
8	Anther dehiscence	Longitudinal			
9	No. of anthers/flower	3			
10	No. of staminodes/ flower	2 or 3			
11	Mean no. of pollen grains/ anther	Male flowers		Bisexual flowers	
		2920±347 (L) 4320±239 (M)		2260±159 (L) 3020±234 (L)	
12	Mean no. of pollen grains/ flower	10160 (Male flower) 7540 (Bisexual flower)			
13	Mean no. of ovules/flower	3			
14	Pollen-ovule ratio	1508:1 (Bisexual flower)			
15	Pollen structure	Spinulose and monolete			
16	Pollen size		Male		Bisexual	
			P	E	P	E
		L	41.70±0.20 µm	26.42±0.15 µm	51.36±0.38 µm	33.96±0.26 µm
		M	42.10±0.28 µm	27.93±0.27 µm	52.44±0.43µm	34.25±0.31µm
17	Pollen shape	Prolate			
18	Stigma type	Dry, papillate			
19	Pollen viability (max%)	90.09±0.35 84.09±0.45 (Male)		86.98±0.17 78.12±0.45 (Bisexual)	
20	Fruit type	Capsule; 2 locule dehiscent, one locule indehiscent			
21	Flower-fruit ratio	65:32			
22	Ovule-seed ratio	325:163			
23	Flower closing time	11.00–12.00 pm			

5.2 *Dictyospermum montanum* Wight

Dictyospermum montanum, commonly called 'mountain day flower', is distributed in South India, Sri Lanka, Assam and Indo-China (Fig. 2 B; Fig. 20 A & B). Erect to partly decumbent, annual or perennial branched herb, rooting at the nodes. Leaves sheathing, 5–16 × 3–6 cm, clustered at the apex of branches; petioles 2–3 cm long; sheath 3–4 cm long, ciliate at the fused margins; lamina elliptic–lanceolate, acuminate at apex. Inflorescence terminal and axillary from the uppermost leaves, 8–20 cm long, lax, ovoid thyrses with hooked hairs. Sepals 3, 3–3.2 × 1.5–1.6 mm, free, glabrous, ovate, with a greenish tinge. Petals 3, ovate, glabrous, white, 4–5 × 2–2.5 mm. Stamens 3, heterantherous: one (medial stamen, M) differs significantly from the others with respect to the shape of its anther. The anther of the medial stamen is yellow with a broad connective and is shield shaped with a 3 mm long partly purple-white filament. The other two stamens are arranged flanking this medial stamen and has purple tinged, bithecous, basifixed anthers, differing from each other only in the colour and length of their filament. While one (lateral stamen 1, L1) has a purple coloured 3 mm long filament, the other (lateral stamen 2, L2) has 2 mm long white filament. This species shows reciprocal enantiostyly, where two types of mirror image flowers are seen, one with white filamented stamen always in the anterior position (median) and medial stamen either to the left (left-handed flowers) or to the right (right-handed flowers) (Fig. 21 A & B). The stigma is positioned facing the medial stamen. The left and right handed flowers bloom alternately within each branch on the inflorescence while their number and position remain random in the inflorescence as a whole. The three stamens are arranged slightly off-center to one side and the gynoecium occupies the other. The style, 1 mm in length is slightly curved to the tip so that it faces the medial stamen. Stigma is capitate. Ovary globose-triangular, white, glabrous, 1 × 1 mm, tricarpellary, syncarpous

with 3 ovules. Capsules are globose-triangular, 3 × 3 mm and glabrous (Fig. 21 A-H)

5.2.1 Phenology

5.2.1.1 Population phenology

Dictyospermum montanum is a perennial plant, propagating through both vegetative and sexual means. The plant shows different behavior in different environments. In forest floors alongside streams, the plant grows throughout the year while in areas where it gets dry during summer, the plant completely dries. In the areas where the population is terminated at the end of the year, they germinate back from seeds with the return of favourable conditions.

In the green house, the plants were maintained throughout the year, and were flowering for most part, with proper care.

Under natural conditions flowering was observed from September to January, of which November to December showed peak in flowering.

5.2.1.2 Flowering phenology

It took an average of 15 days, from the initiation of inflorescence for the first flower to bloom. The inflorescence ranges from 8–20 cm and on average an inflorescence produce about 35–90 flowers. Flowers develop in acropetal succession within individual branches. Flower bud took 4–5 days from initiation, to full bloom (Fig. 22 A-F).

5.2.1.3 Intra-floral phenology

In a population, anthesis took place at 9.30 am to 10.00 am, taking about 15–10 minutes for all the flowers within the population to open. On days with higher temperature and lower humidity the flowers opened earlier than on days with lesser temperature and higher humidity. Also in a population, the

flowers exposed to high intensity of light or even direct light opened earlier than those in the shade (Fig. 23 A-E).

Anthers dehisce 10–20 minutes after anthesis, taking longer to dehisce on humid days. Insects visiting even before anther dehiscence manipulate the anthers with their forelimbs and this seems to somewhat hasten the process of anther dehiscence (Table 29). Flowers closed at around 02.00–02.30 pm.

5.2.2 Pollen biology

5.2.2.1 Pollen morphology

Morphologically the pollen grains produced by the three types of stamen showed no significant differences. All the grains were prolate, spinulose and monolete (Fig. 24 A & B). Pollen produced by the purple filamented (L1) stamen was 26.53±0.22 μm and 18.88±0.16 μm, while pollen from the white filamented (L2) stamen was 25.99±0.31 μm and 18.67±0.09 μm and those from the shield shaped (M) stamen was 26.24±0.21 μm and 18.71± 0.07 μm, along the polar (P) and equatorial (E) axes respectively (n=50).

5.2.2.2 Pollen biochemical analysis

The pollen grains stained with I_2KI solution became brownish black, indicating the presence of starch and those stained with Sudan Black became black indicating the presence of lipid. On staining with Coomassie Brilliant Blue, the pollen grains became blue tinged indicating the presence of protein (Fig. 24 F-H).

5.2.2.3 Pollen production

The lateral stamen with white filament (L2) produced significantly more pollen than the other two whereas the pollen production by the lateral stamen with the purple filament (L2) was significantly lower than the medial stamen

(M) ($p<0.05$). Mean number of pollen produced by the purple filamented lateral stamen (L1) was calculated to be 2824±155 and that by the white filamented lateral stamen (L2) was 3446±240 whereas the medial stamen (M) produced 3162±220 pollen.

5.2.2.4 Pollen-ovule ratio

Average pollen production per flower was estimated to be about 9432 pollen grains and each flower produce 3 ovules thus the pollen to ovule ratio is 3144:1.

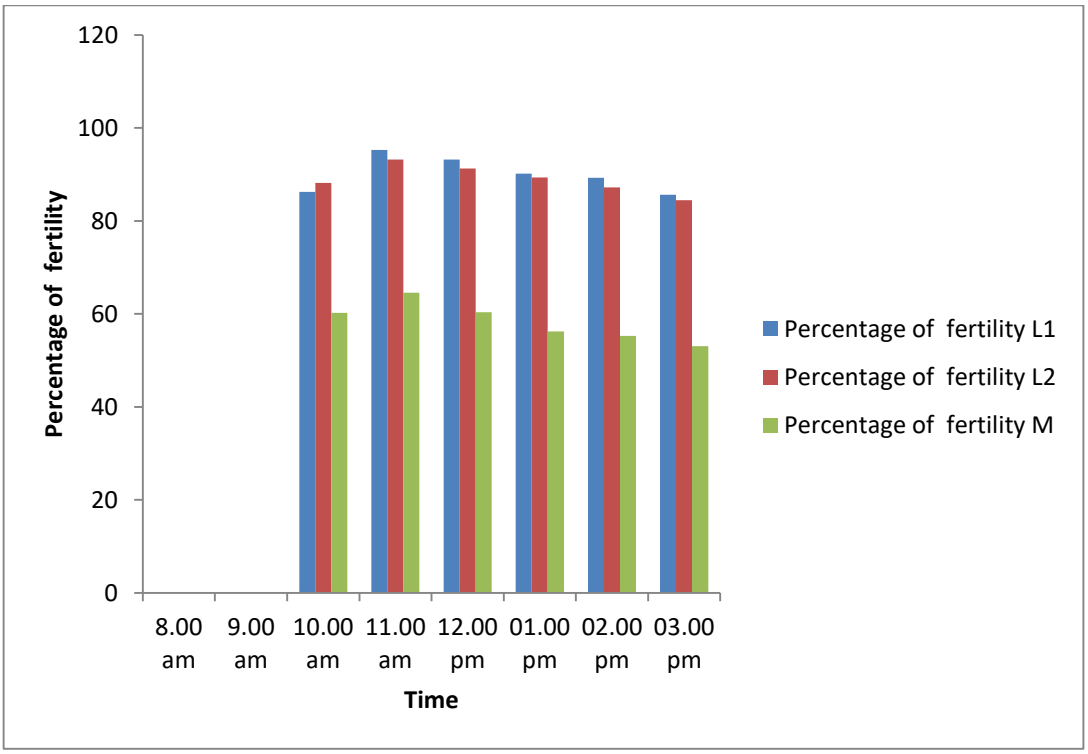
5.2.2.5 Pollen fertility and sterility

Pollen from the lateral stamens was significantly more fertile than those from the medial stamen. Maximum fertile period for all the three stamens were observed around 10.00 am–12.00 pm (Fig. 24 E; table 18; graph 16).

Table 18. *Dictyospermum montanum*: Pollen fertility - acetocarmine test

Sl. No.	Time of observation	Percentage of pollen fertility (mean ±SE)		
		L1	L2	M
1	08.00 am	-	-	-
2	09.00 am	-	-	-
3	10.00 am	86.23±0.32	88.18±0.56	60.24±0.12
4	11.00 am	95.28±0.38	93.20±1.07	64.54±1.29
5	12.00 pm	93.21±0.62	91.26±0.12	60.35±0.62
6	01 00 pm	90.18±0.22	89.34±0.23	56.26±0.51
7	02.00 pm	89.30±0.36	87.22±1.32	55.29±0.48
8	03.00 pm	85.61±0.32	84.46±0.25	53.09±2.2

Graph 16. *Dictyospermum montanum*: Pollen fertility - acetocarmine test



5.2.2.6 Pollen viability

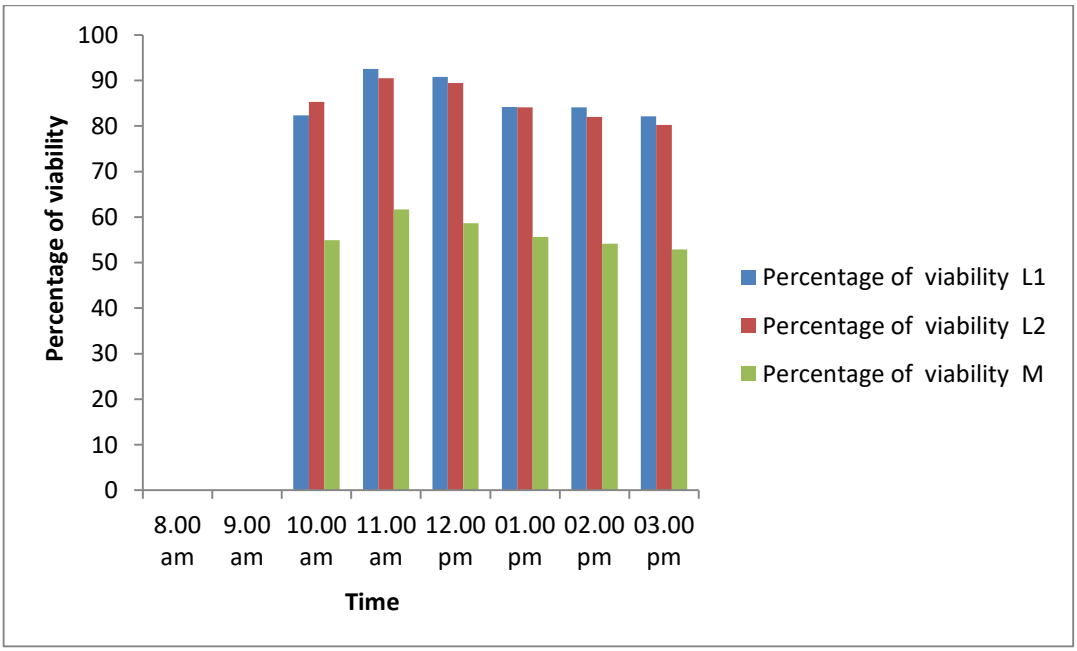
Similar to fertility percentages, pollen from the lateral stamens was significantly more viable than those from the medial stamen.

Maximum viability was observed for all the three were observed (Fig. 24 D; table 19; graph 17) around 10.00 am–12.00 pm (92.58±0.18, 90.50±0.27 and 61.65±1.19 for the L1, L2 and M respectively).

Table 19. *Dictyospermum montanum*: Pollen viability - tetrazolium test

Sl. No.	Time of observation	Percentage of pollen viability (mean ±SE)		
		L1	L2	M
1	08.00 am	-	-	-
2	09.00 am	-	-	-
3	10.00 am	82.38±0.23	85.32±1.2	54.94±0.28
4	11.00 am	92.58±0.18	90.50±0.27	61.65±1.19
5	12.00 pm	90.81±0.44	89.46±0.46	58.65±0.62
6	01 00 pm	84.16±0.22	84.13±0.24	55.66±0.42
7	02.00 pm	84.10±0.65	82.02±0.22	54.19±0.63
8	03.00 pm	82.11±0.39	80.21±0.36	52.92±0.14

Graph 17. *Dictyospermum montanum*: Pollen viability - tetrazolium test



5.2.2.7 Effect of organic and inorganic nutrients on in-vitro pollen germination

In-vitro pollen germination studies showed (Fig. 24 C; table 20; graphs 18 & 19) maximum germination percentage (92.24±0.66%) and maximum pollen

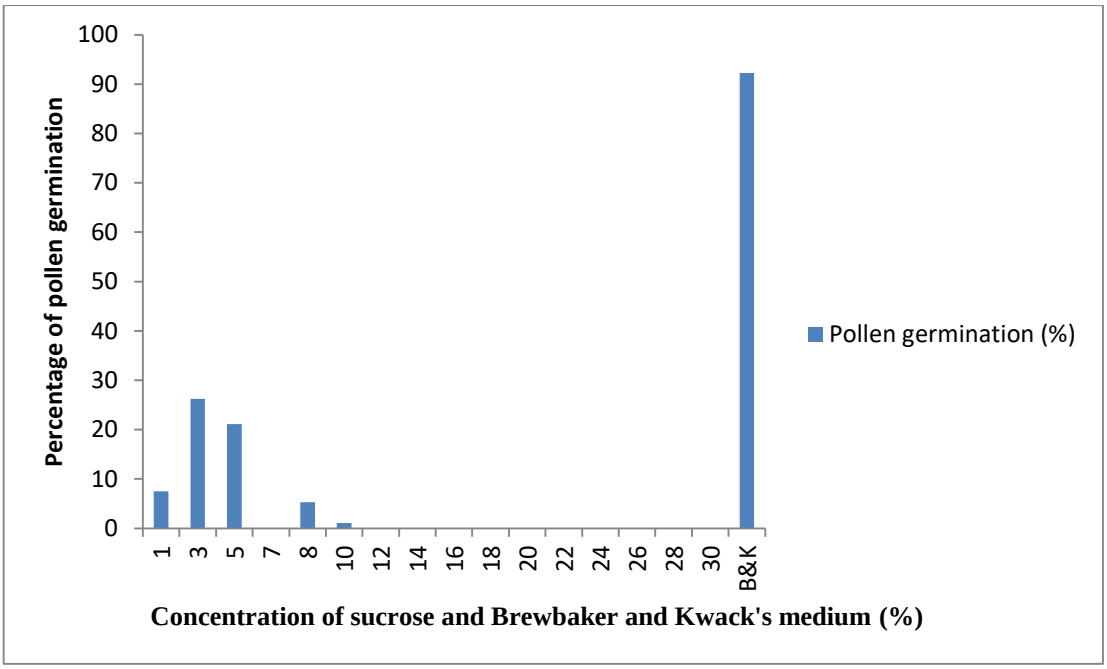
tube length (143.62±3.81 µm) in Brewbaker & Kwack's medium. Sucrose solution of 3% gave the next best result with 26.22±2.6% and 79.32±2.4 µm.

9.32±2.36% pollen germination was observed in calcium nitrate solution (Table 21; graphs 20 & 21) of 300 µg/ml with an average pollen tube length of 42.15±6.0 µm. 100 µg/ml solution gave the maximum percentage of pollen germination (08.76±2.2%) as well as the maximum pollen tube length (36.19±4.7µm) amongst potassium nitrate solutions (Table 22; graphs 22 & 23) of different concentrations. Boric acid solution (Table 23; graphs 24 & 25) of 100 µg/ml gave 07.88±1.1% germination as well 39.07±3.5 µm pollen tube length and magnesium sulphate solution (Table 24; graphs 26 & 27) of 100 µg/ml gave 6.82±2.73% pollen germination and 32.14±7.2 µm pollen tube length.

Table 20. *Dictyospermum montanum*: In-vitro pollen germination in sucrose and Kwack's medium.

Concentration (%) Sucrose	**Pollen germination ± S.E.(%)**	**Pollen tube length ± S.E. (µm)**
1	7.52±1.20	72.54±2.2
3	26.22±2.6	80.01±3.21
5	21.10±2.8	78.36±2.6
8	5.32±1.5	79.62±5.6
10	1.09±0.69	79.32±2.4
15	-	-
20	-	-
25	-	
30	-	-
Brewbaker & Kwack's medium	92.24±0.66	143.62±3.81

Graph 18. *Dictyospermum montanum*: Effect of sucrose and Brewbaker and Kwack's medium - pollen germination



Graph 19. *Dictyospermum montanum*: Effect of sucrose and Brewbaker and Kwack's medium - pollen tube length

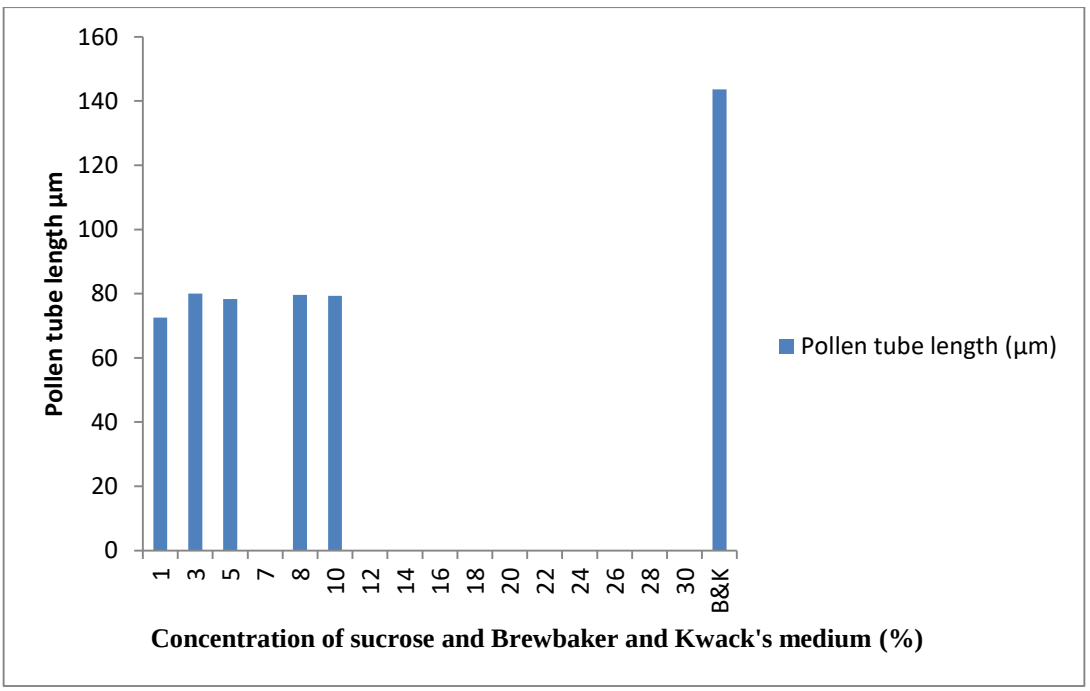
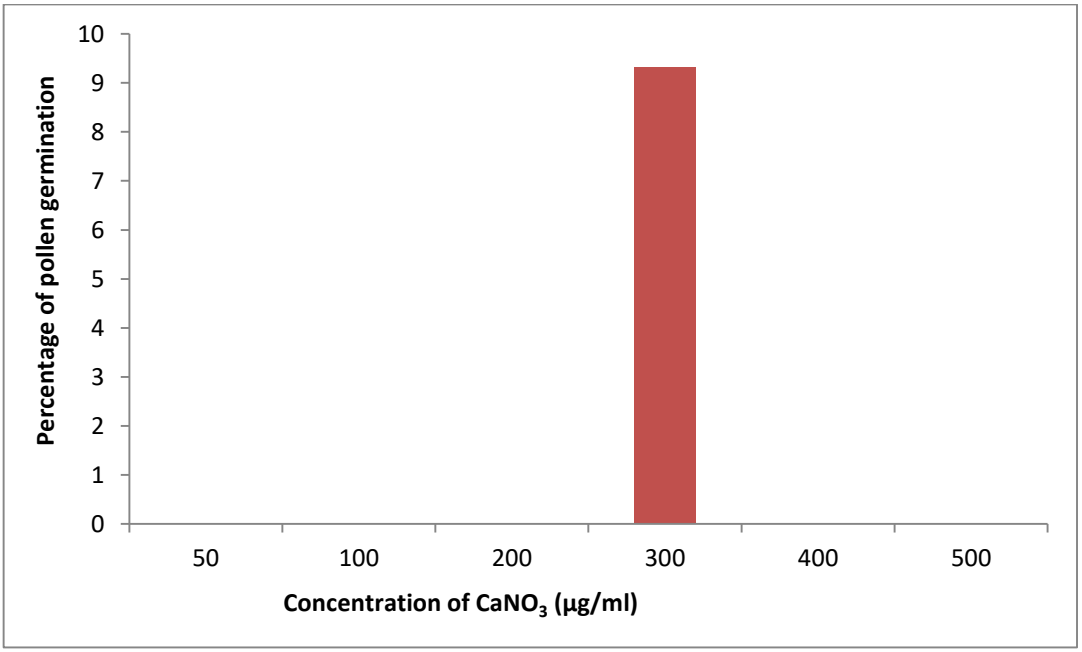


Table 21. *Dictyospermum montanum*: In-vitro pollen germination - effect of calcium nitrate

Sl. No.	Concentration of $CaNO_3$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	50	-	-
2	100	-	-
3	200	-	-
4	300	9.32±2.36	42.15±6.0
5	400	-	-
6	500	-	-

Graph 20. *Dictyospermum montanum*: Effect of calcium nitrate - pollen germination



Graph 21. *Dictyospermum montanum*: Effect of calcium nitrate - pollen tube length

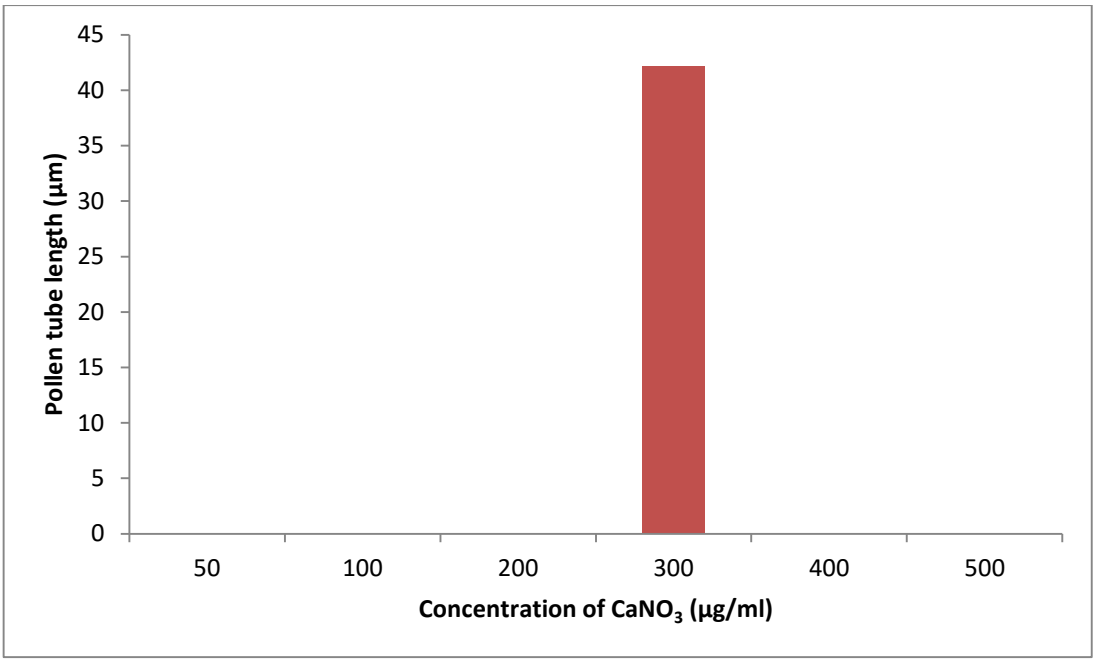
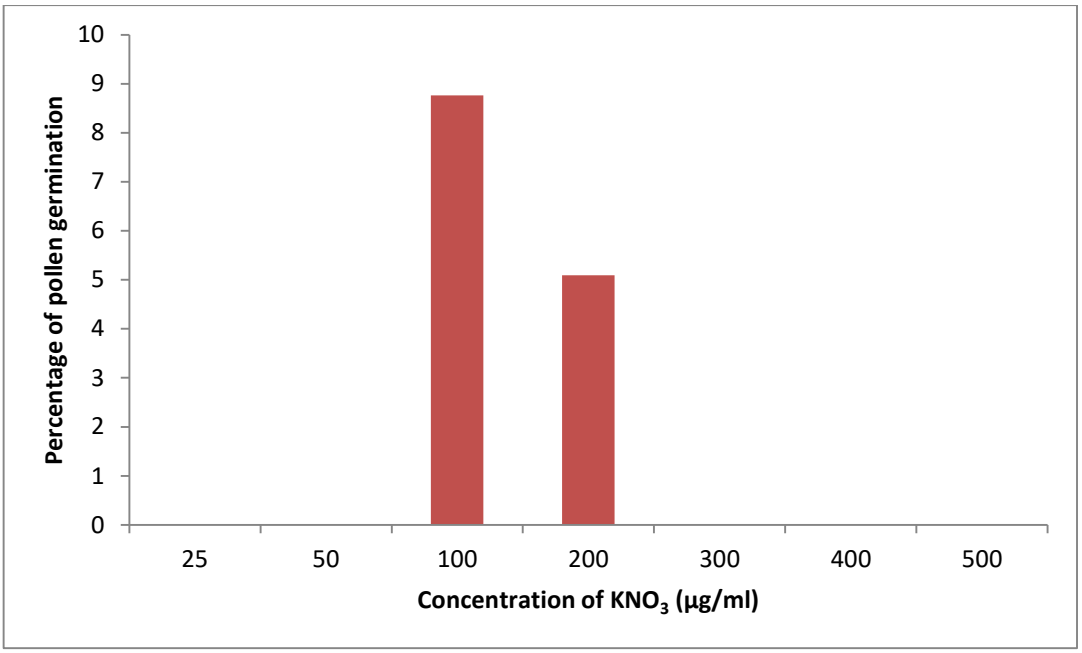


Table 22. *Dictyospermum montanum*: In-vitro pollen germination - effect of potassium nitrate

Sl. No.	Concentration of KNO_3 (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	08.76±2.2	36.19±4.7
4	200	5.09±1.8	34.41±3.2
5	300	-	-
6	400	-	-
7	500	-	-

Graph 22. *Dictyospermum montanum*: Effect of potassium nitrate - pollen germination



Graph 23. *Dictyospermum montanum*: Effect of potassium nitrate - pollen tube length

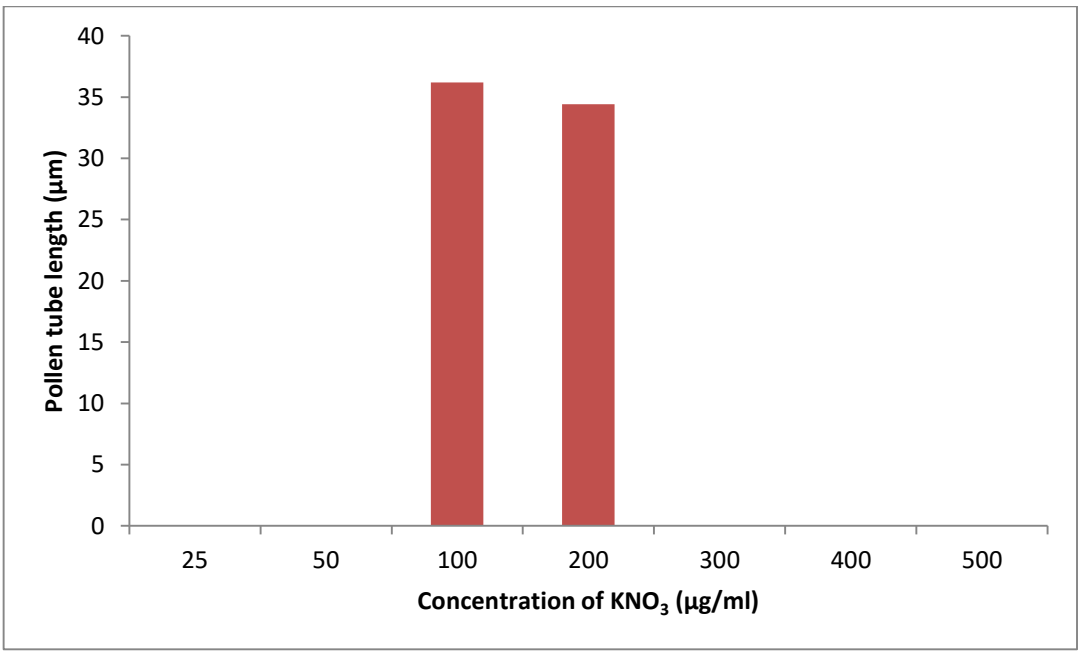
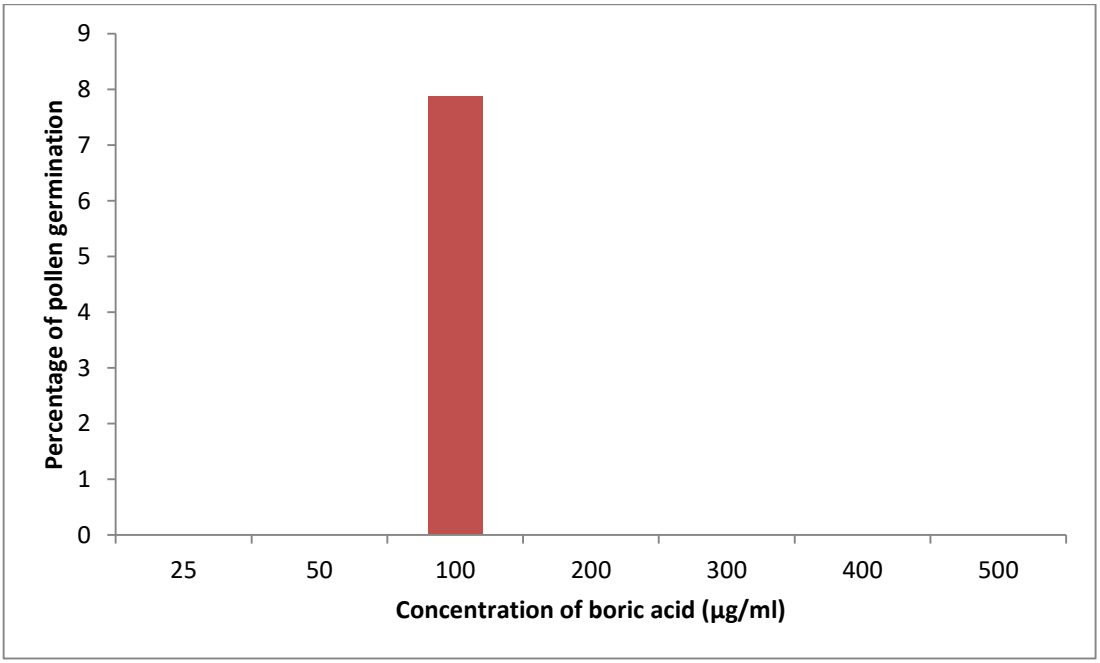


Table 23. *Dictyospermum montanum*: In-vitro pollen germination - effect of boric acid

Sl. No.	Concentration of boric acid (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	07.88±1.1	39.07±3.5
4	200	-	-
5	300	-	-
6	400	-	-
7	500	-	-

Graph 24. *Dictyospermum montanum*: Effect of boric acid - pollen germination



Graph 25. *Dictyospermum montanum*: Effect of boric acid - pollen tube length

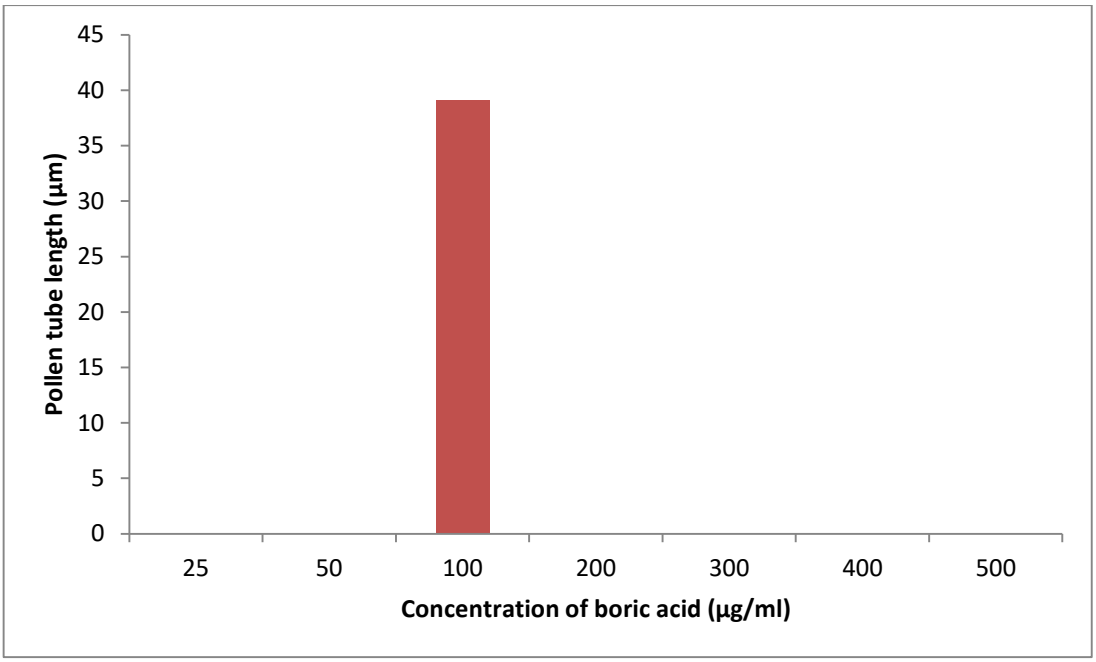
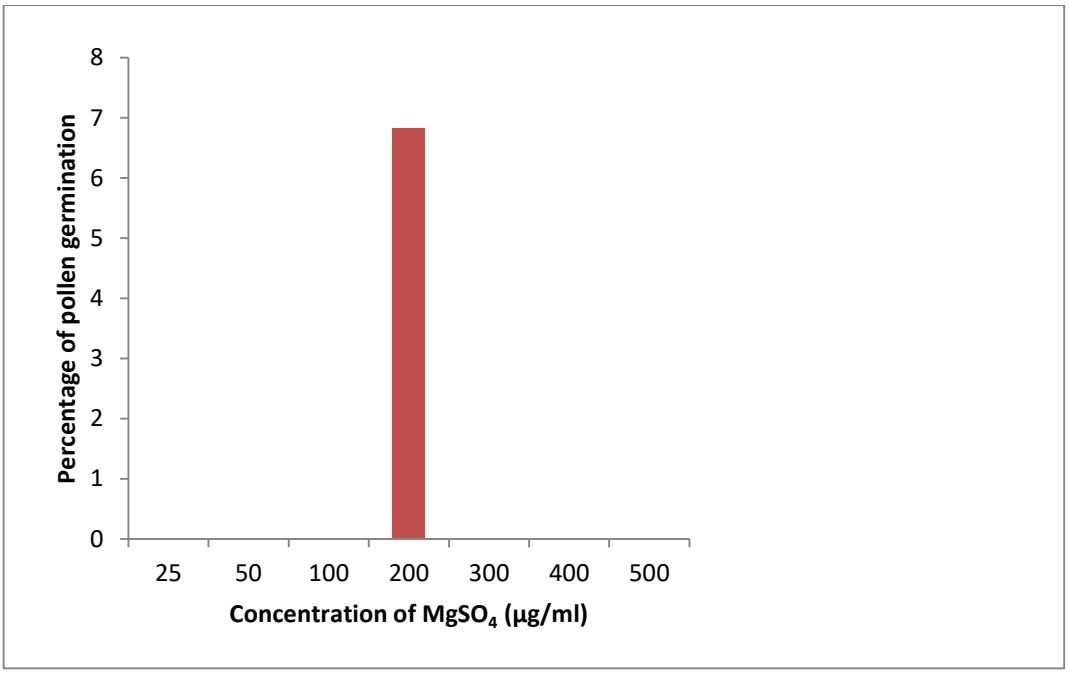


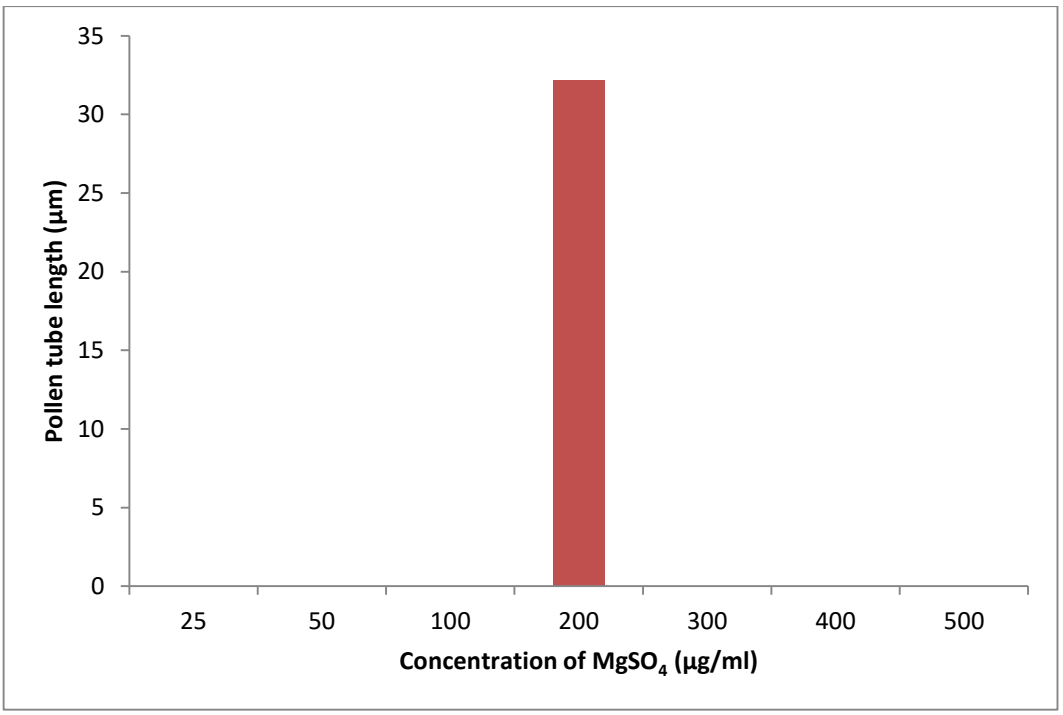
Table 24. *Dictyospermum montanum*: In-vitro pollen germination - effect of magnesium sulphate

Sl. No.	Concentration of $MgSO_4$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	-	-
4	200	6.82±2.73	32.14±7.2
5	300	-	-
6	400	-	-
7	500	-	-

Graph 26. *Dictyospermum montanum*: Effect of magnesium sulphate - pollen germination



Graph 27. *Dictyospermum montanum*: Effect of magnesium sulphate - pollen tube length



5.2.3 Stigma biology

5.2.3.1 Stigma morphology

The stigma is of dry type and the stigmatic surface measures about 73.05±2.6 µm and is covered by papillae of about 46.56±1.76 µm. The style is solid.

5.2.3.2 Stigma receptivity

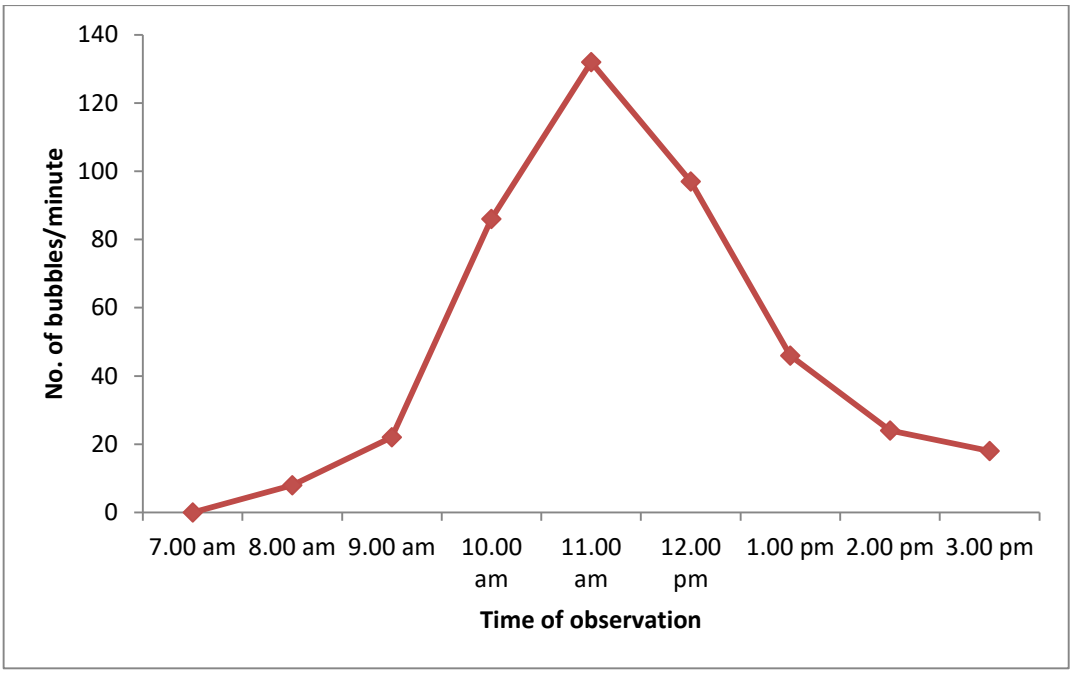
5.2.3.2.1 Stigma receptivity - hydrogen peroxide test

Stigmatic surface showed maximum receptivity around 10.00 am–12.00 pm in hydrogen peroxide solution (Fig. 25 C & D; table 25; graph 28).

Table 25. *Dictyospermum montanum*: Stigma receptivity - hydrogen peroxide test

Sl. No.	Time of observation	No. of bubbles/minute ± S.E.
1	08.00 am	-
2	09.00 am	8±2
3	10.00 am	22±5
4	11.00 am	86±2
5	12.00 pm	132±3
6	01 00 pm	97±1
7	02.00 pm	46±5
8	03.00 pm	24±3
9	08.00 am	18±2

Graph 28. *Dictyospermum montanum*: Stigma receptivity - hydrogen peroxide test



5.2.3.2.2 Cytochemical localization of stigma-surface esterases

Cytochemical localization of stigma-surface esterases using α-naphthyl acetate demonstrated that the stigmatic surface was most receptive during a period between 10.00 am–12.00 pm (Fig. 25 A & B).

5.2.4 Pollination

5.2.4.1 Mode of pollination

Mode of pollination is entomophilous and autogamous.

5.2.4.2 Role of wind in pollination

Examination of the microscopic slides under a microscope revealed an absence of pollen grains thus ruling out wind pollination for the species.

5.2.4.3 Floral visitors and their behaviour

On most days insect visits started soon after anthesis. It was observed that if the anthers had not dehisced by then, the insects left soon without spending much time, only to return once the anthers dehisced. Some of the visitors (*Tetragonula iridipennis* (Apidae) and *Ceratina* sp. (Apidae)), sometimes manipulated the undehisced anthers with their limbs to yield pollen.

The flowers were visited by (Fig. 26–30) *Tetragonula iridipennis*, *Ceratina* sp., *Amegilla* sp. (Apidae), *Halictus* sp. (Halictidae), *Salpinogaster punctifrons*, *Apies cerena* (Apidae), *Osmia* sp. (Apidae), *Nomia* sp. (Halictidae), different types of syrphus flies (Syrphidae), *Lucilia* sp. (Calliphoridae, blow fly) and *Anthochoris* sp. (Anthochoridae bug). Among them, all except *Lucilia* sp. and *Anthochoris* sp. were identified as effective pollinators.

Tetragonula iridipennis had the highest rate of stigma touch and high frequency of visits. In a single visit, *T. iridipennis* foraged on multiple flowers, randomly going from one flower to the other and repeating visits multiple times. The time spent on each flower decreased with the advent of time. It would land straight on the anthers in a flower and move around within the flowers, manipulating the anthers and feeding on pollen. In majority of instances insects went straight on to the medial stamens. During these maneuvers it gets dusted with pollen, especially on the ventral and lateral abdomen, dorsal thorax and head and when it moves on to the next flower, the pollen gets transferred on to their stigmas.

Ceratina sp., *Halictu*s sp., and *Apies cerena* shows similar behavioral patterns. *Ceratina* sp. and *Apies cerena* hovers over a flower for a few seconds before landing and the time spent on flowers, foraging, also varies. The syrphus flies do not land on the flowers but works on the anthers by

hanging on to the stamens and sometimes miss making contact with the stigma. *Lucilia* sp. works on the anthers from the top, foraging for pollen, and completely misses the stigma, whereas the bug (*Anthochoris* sp.) eats the anthers and sometime the entire flowers. More details on the floral visitors are given in the table 26.

Table 26. *Dictyospermum montanum*: Floral visitors and their behaviours

Sl.No.	Name of the taxa with family	Nature	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality		
							Field 1	Field 2	CU campus
1	*Tretragonula iridipennis* Apidae	Pollinator	10.45 am-2.30 pm	5-40 seconds	+++	High	✓	✓	✓
2	*Amegill*a sp. Apidae	Pollinator/ visitor	10.00 am-02.00 pm	1-5seconds	++	High	✓	✓	✓
3	*Ceratina* sp. Apidae	Pollinator	11.00 am-01.00 pm	5-20 seconds	++	High	✓	✓	✓
4	*Osmia* sp. Apidae	Pollinator	11.00 am-01.00 pm	5-25seconds	+	High	✓	✓	✓
5	*Salpinogaster punctifrons* Syrphidae	Pollinator/ visitor	11.30 am-01.30 pm	3-10seconds	++	Intermediate	✓	✓	-
6	Unidentified sp. 2 Syrphidae	Pollinator	12.00 pm-01.30 pm	4-10 seconds	++	Intermediate	✓	-	-
7	Unidentified sp. 3 Syrphidae	Pollinator	12.00 pm-01.00 pm	3-10 secconds	++	Low	✓	-	-
8	*Apis cerena* Apidae	Pollinator	11.30 pm-01.30 pm	3-12 seconds	++	Intermediate	-	✓	-
9	Unidentified sp. 4 Syrphidae	Pollinator	11.30 am-01.30 pm	3-8 seconds	++	Low	✓	-	-
10	Unidentified sp. 5 Syrphidae	Pollinator	12.00 pm-01.00 pm	3-10 seconds	+++	Intermediate	-	-	✓

Sl.No.	Name of the taxa with family	Nature	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality		
							Field 1	Field 2	CU campus
11	Unidentified sp. 6 Syrphidae	Pollinator	11.30 am-02.00 pm	5-10 seconds	++	Low			✓
12	*Halictus* sp. Halictidae	Pollinator	11.00 am -02.00 pm	5-15 seconds	+++	Intermediate	✓	✓	
13	*Nomia* sp. Halictidae	Pollinator	11.30 am-01.30 pm	5-20 seconds	++	Low	✓		✓
14	*Lucilia* sp. Calliphoridae	Pollen robber	10.30 am 11.00 am	10-20 seconds	-	Low	✓		
15	*Anthocoris* sp. Anthochoridae	Predator	Random	-	-	-	-	-	-

Stigma touch: +++ very good; ++ good; + poor

Frequency of visits: High (5–30 visits/day); Intermediate (1–5 visits/day); Low (<1 visit/day).

5.2.4.2 Pollination efficiency

After the flowers closed, their stigmas were observed under a microscope. Stigmas of 46% of flowers showed the presence of pollen.

To study the pollination efficiency by individual visitors, stigmas were observed right after the first visit. It was found that *Tetragonula iridipennis* (Fig. 26) is the most efficient pollinator of *D. montanum*.

5.2.5 Breeding system

The fruit set and seed set percentages from each of the pollination treatments are shown in the tables 27 & 28 and graphs 29 & 30.

5.2.5.1 Apomixis

No fruit set was observed in flowers bagged after emasculation, prior to anthesis.

5.2.5.2 Autogamy

Flowers bagged before anthesis showed 5% fruit set in population 1 and no fruit set in population 2.

5.2.5.3 Manual self-pollination (MSP)

Pollinating flowers by pollen from the same flowers gave 52.5% of fruit set in population 1 and 48.00% in population 2.

5.2.5.4 Manual cross-pollination (MCP)

Flowers pollinated by pollen from other flowers showed 77.50% of fruit set in population 1 and 76.00% of fruit set in population 2.

5.2.5.5 Open pollination (OP)

Flowers left to be pollinated by natural pollinators under natural conditions showed 47.50% of fruit set in population 1 and 44.00% in population 2.

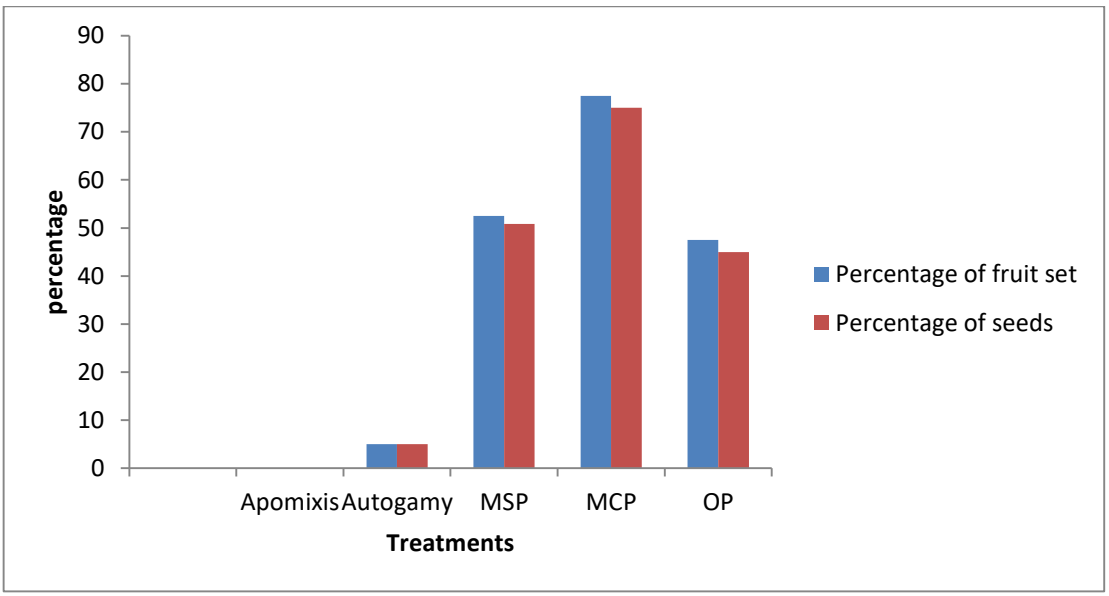
Table 27. *Dictyospermum montanum*: Breeding system, Population 1

Sl. No.	Treatments	POP 1Vellanipacha					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seeds
1	Apomixis	40	0	0	0	3	0
2	Autogamy	40	2	5	6	3	5
3	MSP	40	21	52.5	61	3	50.83
4	MCP	40	31	77.5	90	3	75.00
5	OP	40	19	47.5	54	3	45.00

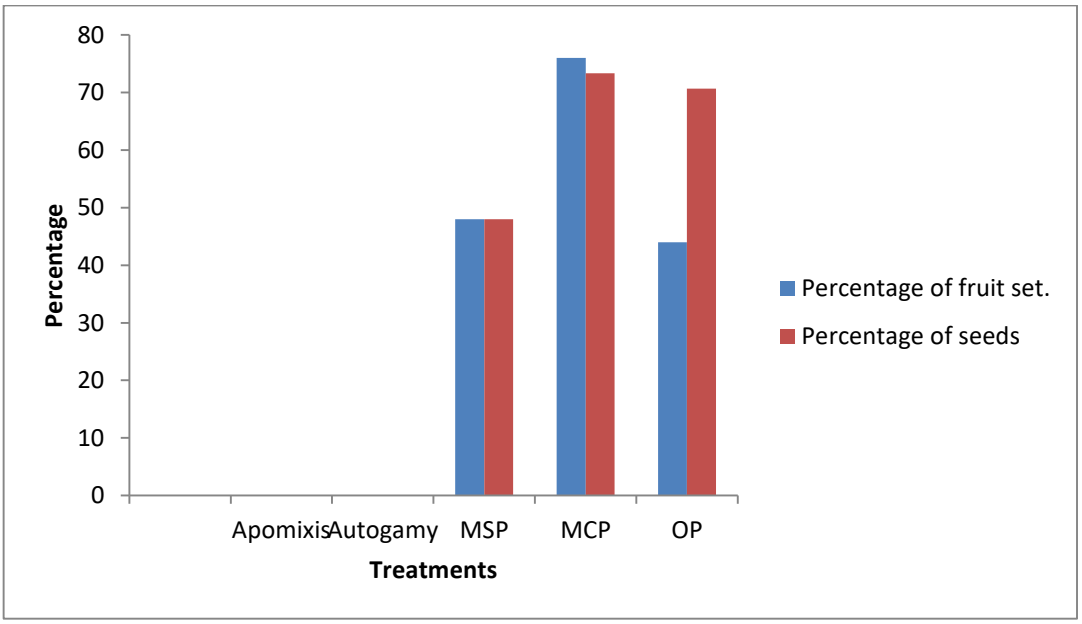
Table 28. *Dictyospermum montanum*: Breeding system, Population 2

Sl. No.	Treatments	POP 2 Adivaram					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seeds
1	Apomixis	25	0	0	0	3	0
2	Autogamy	25	0	0	0	0	0
3	MSP	25	12	48.00	36	3	48
4	MCP	25	19	76.00	55	3	73.33
5	OP	25	11	44.00	53	3	70.67

Graph 29. *Dictyospermum montanum*: Breeding system - fruit set and seed set at population 1



Graph 30. *Dictyospermum montanum*: Breeding system - fruit set and seed set at population 2



5.2.6 Fruit and seed biology

After fertilization the petals fall off from the flower but the sepals persists on the fruit. The fruits matured 34 days after fertilization and the capsules dehisced 6–8 days after fertilization. Capsules are dry-dehiscent, globose, glabrous and trilocular. The capsules dehisce along the septa (Fig. 23 F-H).

Seeds 3, rarely 2, light-dark brown, hemispherical, dorsal surface convex. Hilum is linear, placed in a furrow on the lateral surface. Seed surface ornamented with irregular ridges radiating from the embryotega. The ornamentations show primary polygonate cells with elevated edges, fused crests and depressed faces while the secondary cells are also polygonate with slightly depressed edges and elevated faces.

5.2.6.1 Flower-fruit ratio and ovule-seed ratio

Under natural conditions, the flower to fruit set ratio was 13:6 and the ovule to seed set ratio was 195:105.

5.2.6.2 Seed germination

Seeds kept under laboratory conditions did not germinate but the seeds kept in pots under nursery conditions showed 11% germination (Fig. 31).

Table 29. *Dictyospermum montanum*: Summary of floral characters

Sl. No.	Floral characters	Observations		
1	Flowering period	September to January		
2	Flower type	Zygomorphic, bisexual		
3	Flower colour	White		
4	Odour	Absent		
5	Nectar	Absent		
6	Anthesis time	9.30-10.00 am		
7	Anther dehiscence time	9.40-10.20 am		
8	Anther dehiscence	Longitudinal		
9	No. of anthers/flower	3		
10	No. of staminodes/flower	0		
11	Mean no. of pollen grains/ anther	2824±155 (L1) 3446±240 (L2) 3162±220 (M)		
12	Mean no. of pollen grains/ flower	9432		
13	Mean no. of ovules/flower	3		
14	Pollen-ovule ratio	3144:1		
15	Pollen structure	Spinulose and monolete		
16	Pollen size		P	E
		L1	26.53±0.22µm	18.88±0.16µm
		L2	25.99±0.31µm	18.67±0.09µm
		M	26.24±0.21µm	18.71±0.07µm
17	Pollen shape	Prolate		
18	Stigma type	Dry, papillate		
19	Pollen viability (max%)	92.58±0.18 (L1) 90.50±0.27 (L2) 61.65±1.19 (M)		
20	Fruit type	Capsule		
21	Flower-fruit ratio	13:6		
22	Ovule-seed ratio	195:105		
23	Flower closing time	02.00–02.30 pm		

5.3 *Floscopa scandens* Lour.

Floscopa scandens, 'the climbing flower cup', is commonly found in marshes and on the sides of streams (Fig. 2 C; Fig. 32 A & B). It is also grown as an aquarium plant.

Extensively spreading, decumbent, perennial herbs. Stems branched, terete, green with a purplish tinge at the nodes. Leaves spirally arranged, strongly reduced below the inflorescence; sheath 0.6–0.8 cm long, pubescent, long ciliate at the fused margin; lamina 3.5–4.4 × 1–1.3 cm, lanceolate–elliptic, acute to acuminate at apex. Inflorescence compound, more or less sessile, terminal thyrses, and smaller ones from the axil of reduced distal leaves, compact to moderately lax. Flowers bisexual, sometimes male. Sepals 2 mm long, purple, densely glandular hairy. Petals purplish-lilac, upper two elliptic, acute at apex, lower one oblong, rounded at apex. Stamens 6, dimorphic; filaments fused into groups of 3 at the base. Anthers of the upper 3 stamens (US) with a broad connective and small distal yellow anther sacs while those of the lower 3 stamens (LS) with a narrow whitish connective and larger, purplish anther sacs. Ovary glabrous; style long, slender, pink; stigma capitate, papillate. Capsules 2–3 mm long, dorsiventrally compressed, ellipsoid. Seeds hemispherical–elliptic (semi-lunar) in outline, greyish with a tinge of purple, 12−16 ribbed, farinose (Fig. 33 A-G).

5.3.1 Phenology

5.3.1.1 Population and seasonal phenology

The plant propagates through seeds and spreads by rooting at the nodes. Plants flourish alongside streams and they perennate through seasons.

Flowering was observed from December to March in natural populations, January to February being the peak.

5.3.1.2 Flowering phenology

Inflorescence starts flowering, 6–9 days after its initiation and produced about 55–80 flowers. Flowering takes place in an acropetal manner in each cincinnus (Fig. 34 A-H).

5.3.1.3 Intra-floral phenology

Anthesis takes place around 10.20 to 10.40 am. The flowers remain open for 4–4.5 hours. Anther dehisces 5–10 minutes after anthesis (Table 41). The petals deliquesced and the flowers closed by 02.30 pm (Fig. 35 A-G).

5.3.2 Pollen biology

5.3.2.1 Pollen morphology

Pollen grains are spinulose and monosulcate (Fig. 36 A & B). Pollen produced by the two types of anthers (US and LS) does not show any significant differences ($p>0.05$) (Fig.). Pollen produced by the lower stamens (LS) measure 35.31±0.70 μm (P) and 21.26±0.5 μm (E); pollen produced by the upper stamens measure 36.18±0.35 μm (P) and 21.45±0.33 μm (E). (n=50).

5.3.2.2 Pollen biochemical analysis

The pollen grains stained with I_2KI solution became brownish black, indicating the presence of starch and those stained with Sudan black became black indicating the presence of lipid. On staining with Coomassie Brilliant Blue, the pollen grains became blue tinged indicating the presence of protein (Fig. 36 F-H).

5.3.2.3 Pollen production

Pollen production by the two types of stamen showed no significant difference ($p>0.05$). A single lower stamen produced an average of 611±81 and a single upper stamen produced an average of 308±66 pollen grains.

5.3.2.4 Pollen-ovule ratio

Average pollen production per flower was estimated to be about 2757 pollen grains and each flower produce 3 ovules thus the pollen to ovule ratio is 919:1.

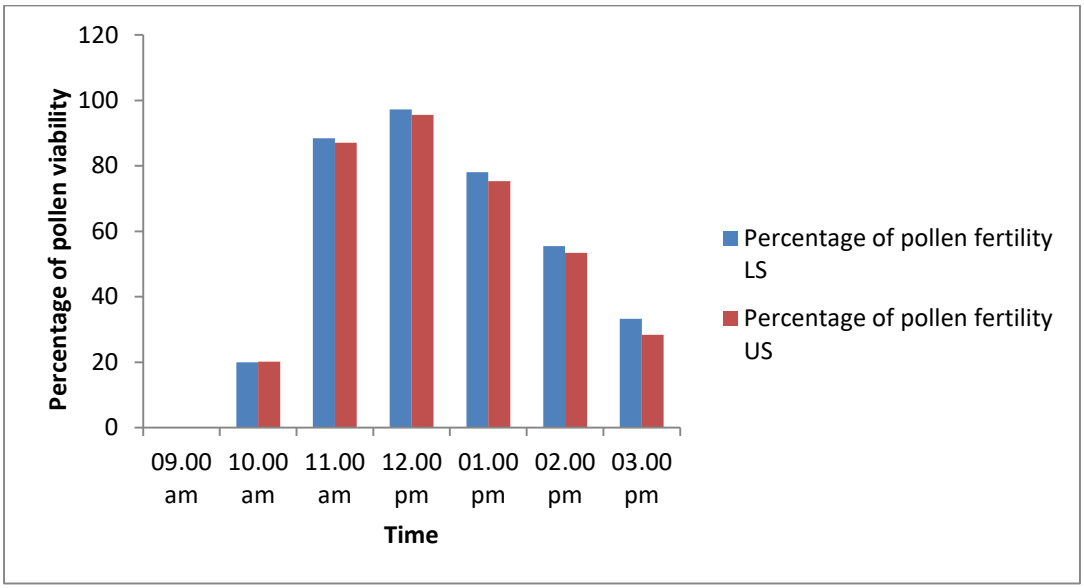
5.3.2.5 Pollen fertility and sterility

Acetocarmine staining technique showed maximum fertility (Fig. 36 D; table 30; graph 31) at around 11.00 am–12.00 pm (97.28±0.73% and 95.62±0.56% for LS and US respectively).

Table 30. *Floscopa scandens*: Pollen fertility - acetocarmine method

Sl No.	Time of observation	Percentage of pollen fertility±S.E	
		LS	US
1	09.00 am	-	-.
2	10.00 am	19.91±1.87	20.17±1.39
3	11.00 am	88.43±1.20	87.08±1.53
4	12.00 pm	97.28±0.73	95.62±0.56
5	01.00 pm	78.07±0.21	75.35±0.74
6	02.00 pm	55.49±.30	53.38±0.92
7	03.00 pm	33.22±0.41	28.39±0.91

Graph 31. *Floscopa scandens*: Pollen fertility - acetocarmine test



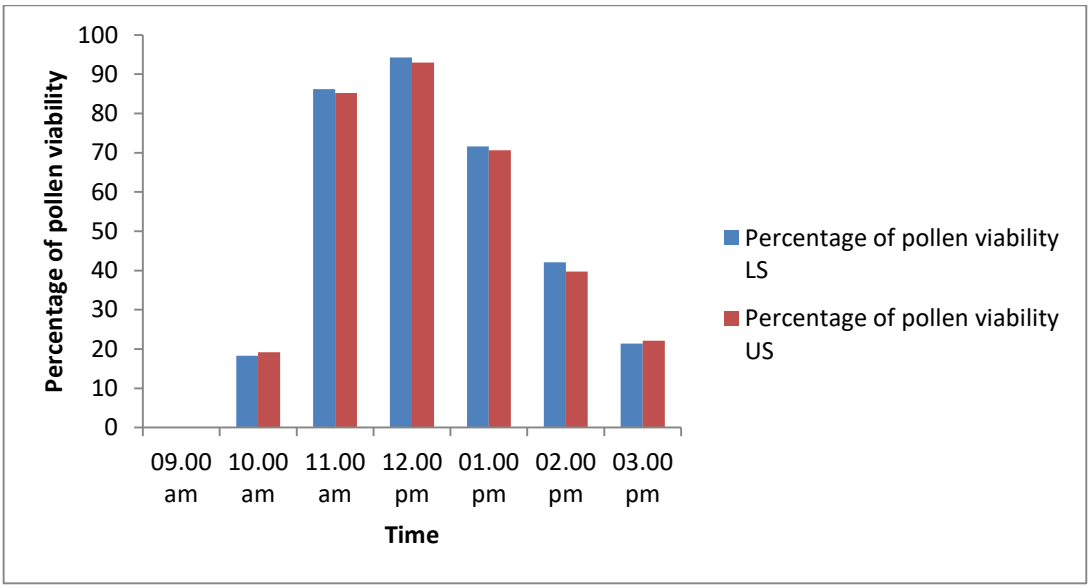
5.3.2.6 Pollen viability

The stamens showed no significant differences in their viability (Fig. 36 C; table 31; graph 32). Maximum viability was observed around 11.00 am–12.00 pm (94.26±0.63% and 92.93±0.96% for LS and US respectively) and then steadily decreased.

Table 31. *Floscopa scandens*: Pollen viability - tetrazolium method

Sl. No.	Time of observation	Percentage of pollen viability±S.E	
		LS	US
1	09.00 am	-	-.
2	10.00 am	18.32±2.31	19.21±1.68
3	11.00 am	86.18±0.92	85.22±1.60
4	12.00 pm	94.26±0.63	92.93±0.96
5	01.00 pm	71.57±0.72	70.62±0.84
6	02.00 pm	42.09±1.23	39.74±0.51
7	03.00 pm	21.49±0.54	22.12±0.11

Graph 32. *Floscopa scandens*: Pollen viability - tetrazolium test



5.3.2.7 Effect of organic and inorganic nutrients on in-vitro pollen germination

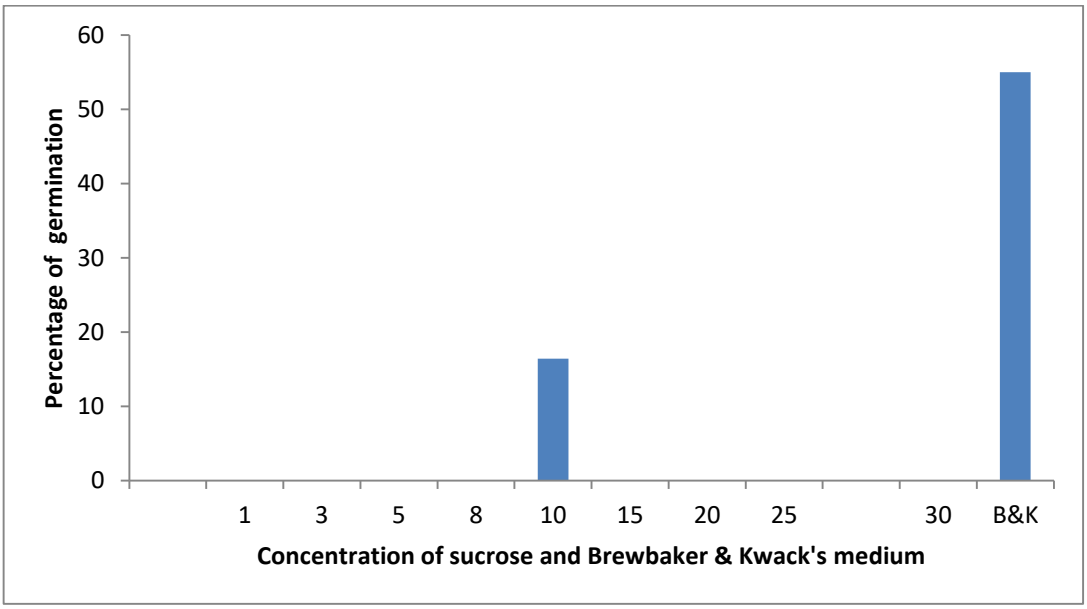
In-vitropollen germination studies (Fig. 36 E; table 32; graphs 33 & 34) showed germination in 10% sucrose solution (16.42±3.26% germination and 72.12±5 µm pollen tube length) and in Brewbaker & Kwack's medium (55.03±3.92 % germination and 129.61±4.78 µm pollen tube length).

Calcium nitrate solution (Table 33; graphs 35 & 36) of 300 µg/ml gave 5.28±1.39% of germination and 48.22±3.9 µm pollen tube length. 100 µg/ml of potassium nitrate solution (Table 34; graphs 37 & 38) gave 04.6±1.23% of pollen germination and 39.76±3.6 µm of pollen tube length. While boric acid solution (Table 35; graphs 39 & 40) of 100 µg/ml gave the maximum germination percentage (05.82±0.53%), maximum pollen tube length was obtained in a solution of 100 µg/ml (31.45±4.63 µm) whereas magnesium sulphate solution (Table 36; graphs 41 & 42) of 200 µg/ml gave 5.52±2.23% germination and 36.24±5.25 µm pollen tube length.

Table 32. *Floscopa scandens*: In-vitro pollen germination in sucrose and Kwack's medium.

Concentration (%) Sucrose	Pollen germination ± S.E.(%)	Pollen tube length ± S.E. (µm)
1	-	-
3	-	-
5	-	-
8	-	-
10	16.42±3.26	72.12±5.8
15	-	-
20	-	-
25	-	
30	-	-
Brewbaker & Kwack's medium	55.03±3.92	129.61±4.78

Graph 33. *Floscopa scandens*: Effect of sucrose and Brewbaker and Kwack's medium - pollen germination



Graph 34. *Floscopa scandens*: Effect of sucrose and Brewbaker and Kwack's medium - pollen tube length

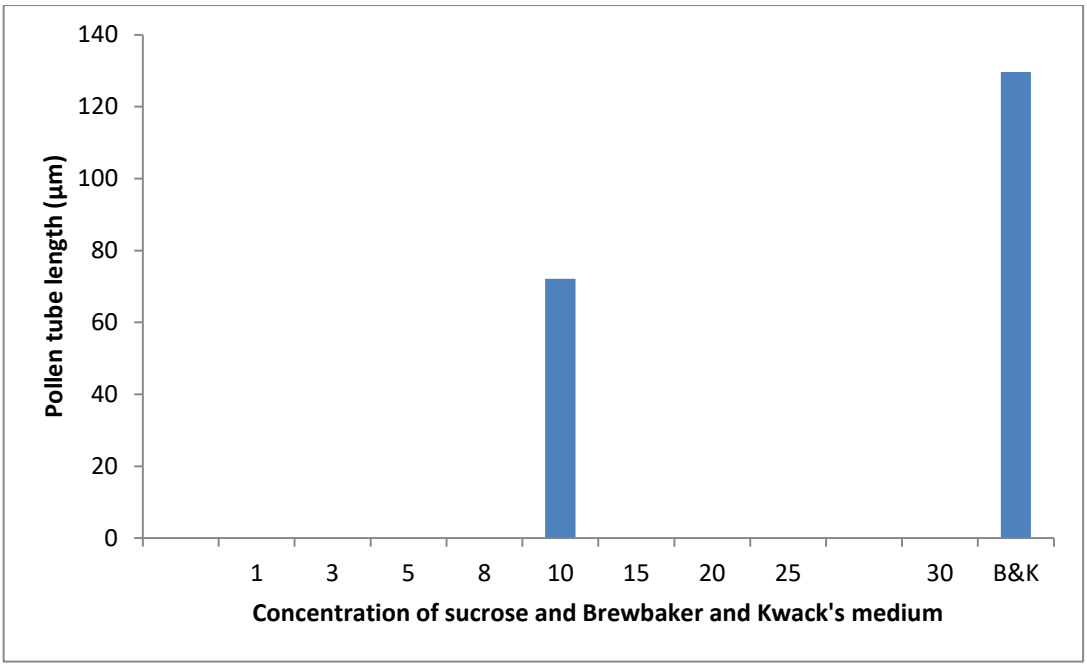
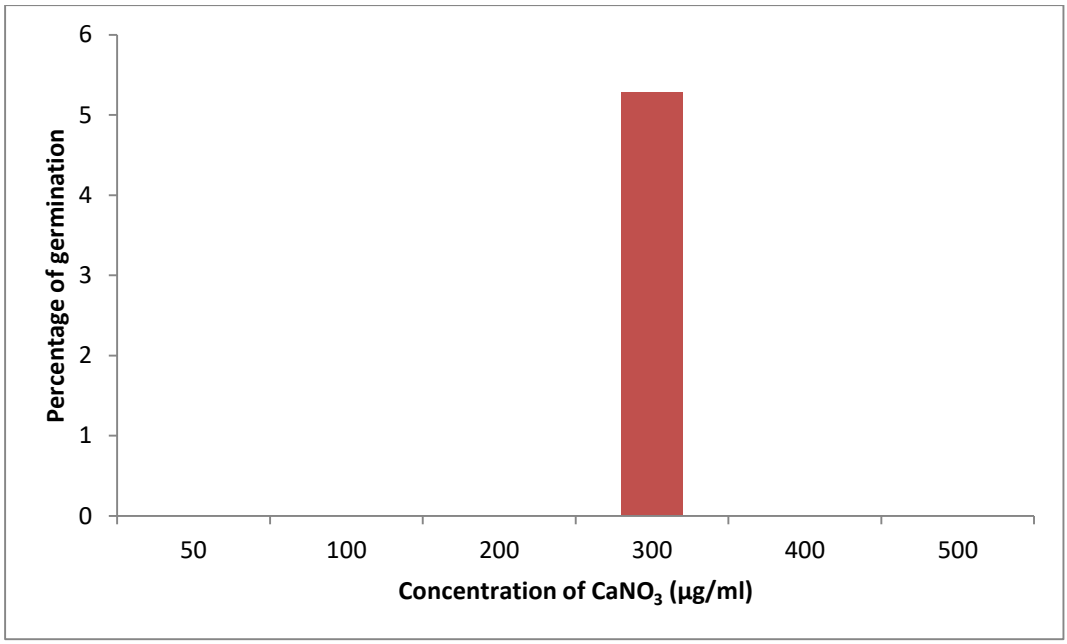


Table 33. *Floscopa scandens*: In-vitro pollen germination - effect of calcium nitrate

Sl. No.	Concentration of $CaNO_3$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	50	-	-
2	100	-	-
3	200	-	-
4	300	5.28±1.39	48.22±3.9
5	400	-	-
6	500	-	-

Graph 35. *Floscopa scandens*: Effect of calcium nitrate - pollen germination



Graph 36. *Floscopa scandens*: Effect of calcium nitrate - pollen tube length

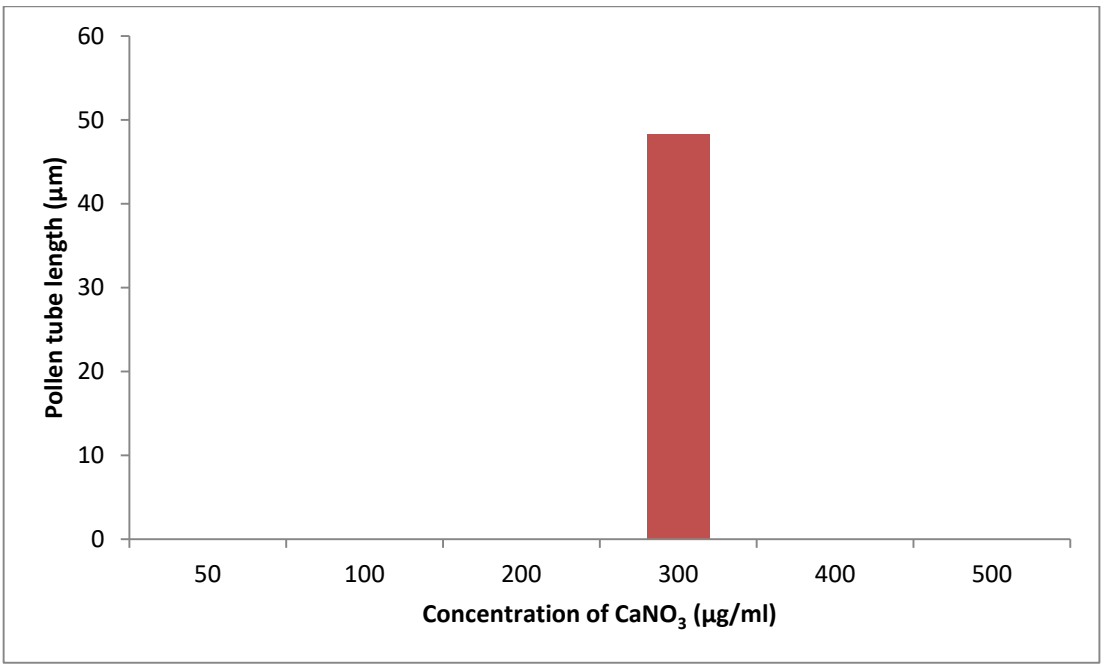
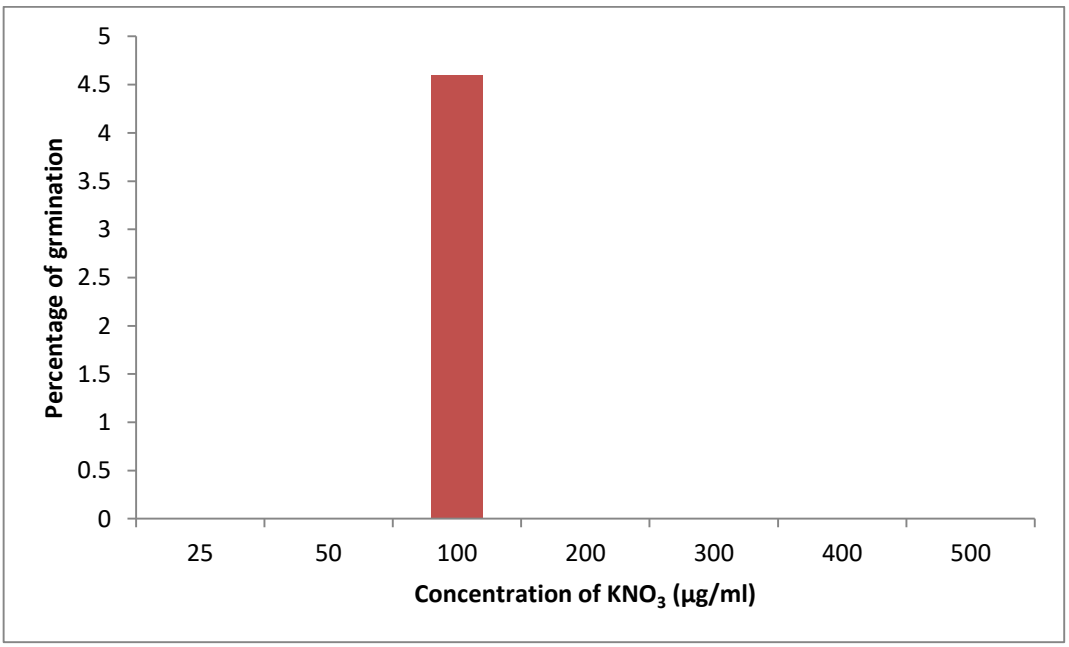


Table 34. *Floscopa scandens*: In-vitro pollen germination - effect of potassium nitrate

Sl. No.	Concentration of KNO_3 (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	04.6±1.23	39.76±3.6
4	200	-	-
5	300	-	-
6	400	-	-
7	500	-	-

Graph 37. *Floscopa scandens*: Effect of potassium nitrate - pollen germination



Graph 38. *Floscopa scandens*: Effect of potassium nitrate - pollen tube length

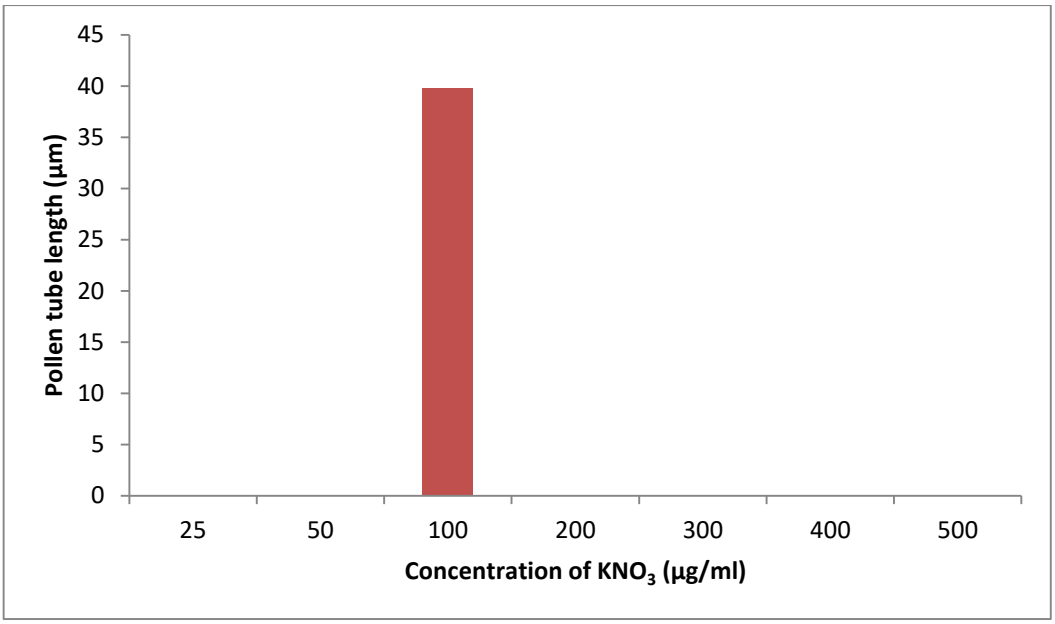
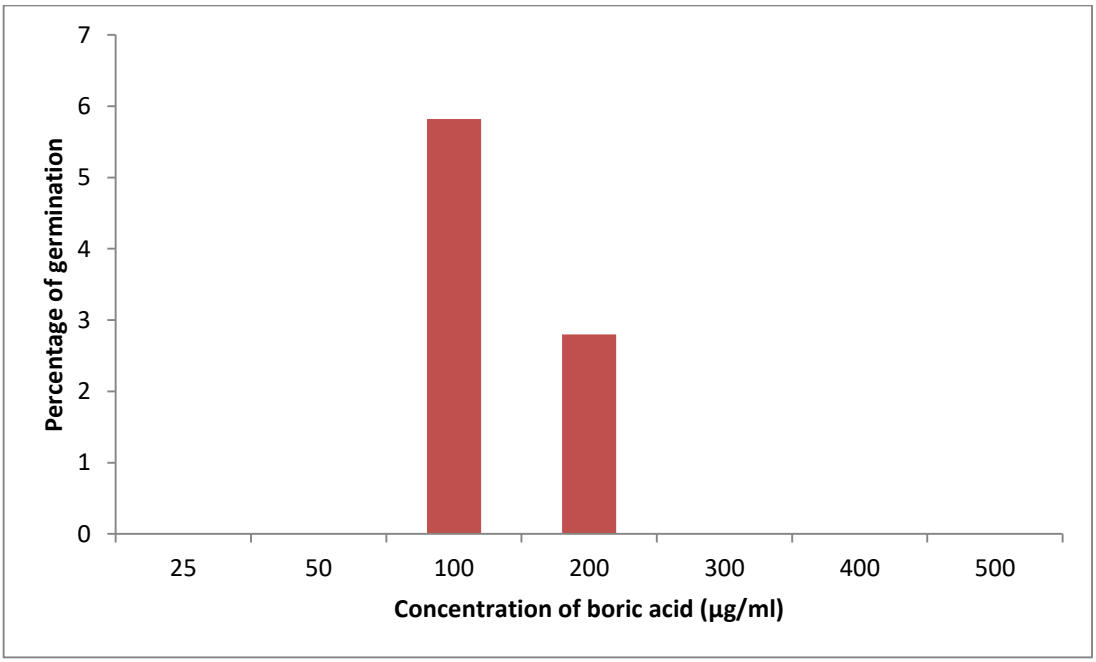


Table 35. *Floscopa scandens*: In-vitro pollen germination - effect of boric acid

Sl. No.	Concentration of boric acid (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	05.82±0.53	22.57±4.12
4	200	2.8±4.26	31.45±4.63
5	300	-	-
6	400	-	-
7	500	-	-

Graph 39. *Floscopa scandens*: Effect of boric acid - pollen germination



Graph 40. *Floscopa scandens*: Effect of boric acid - pollen tube length

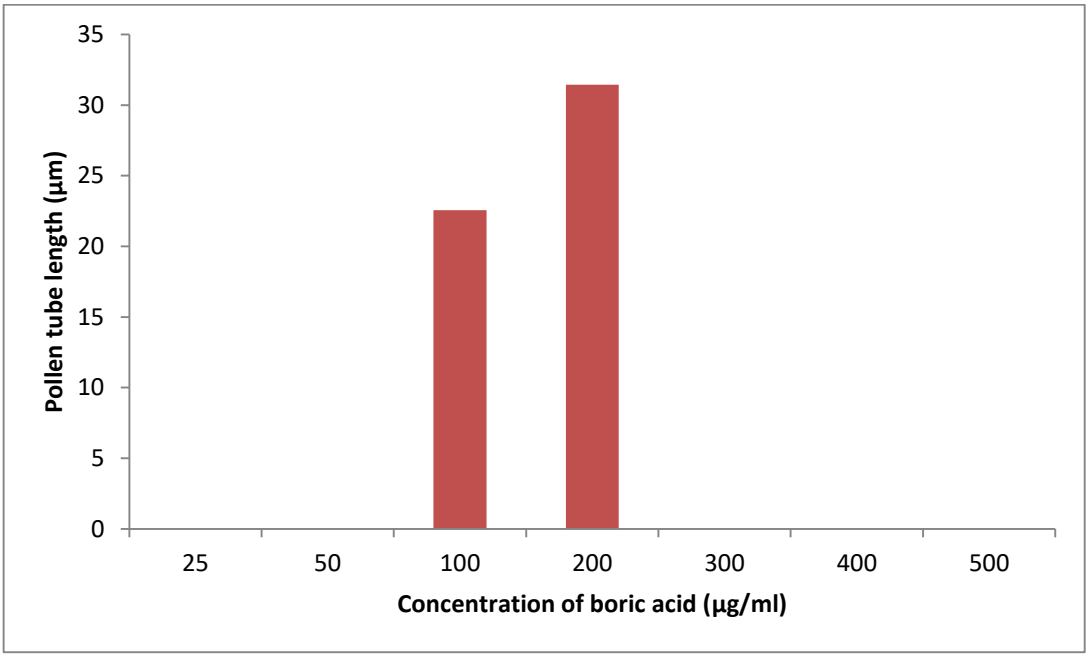
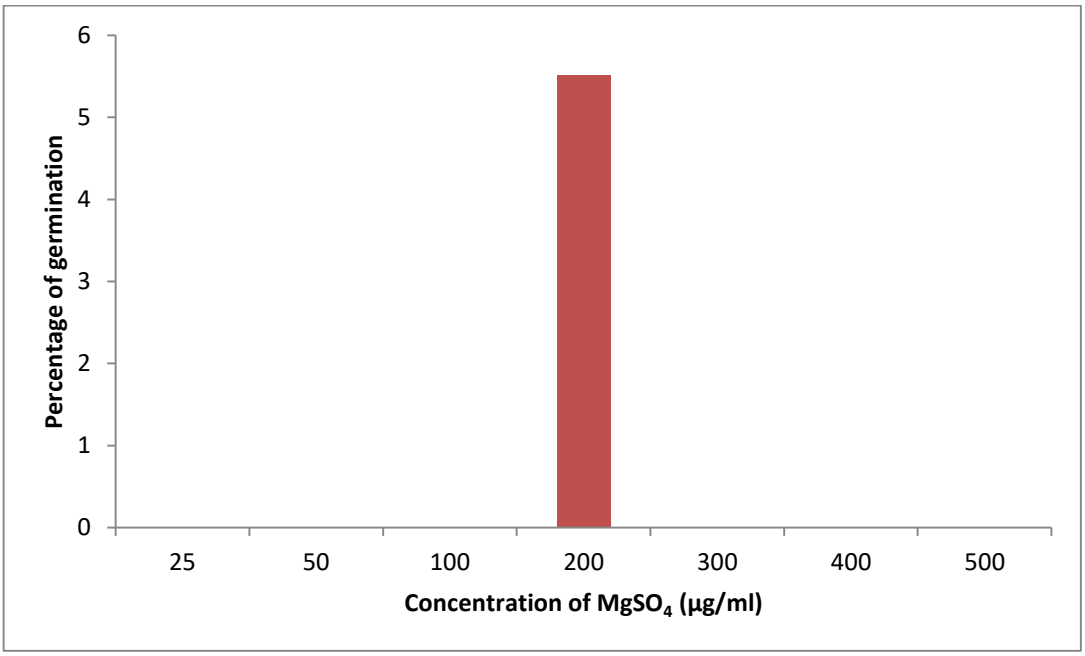


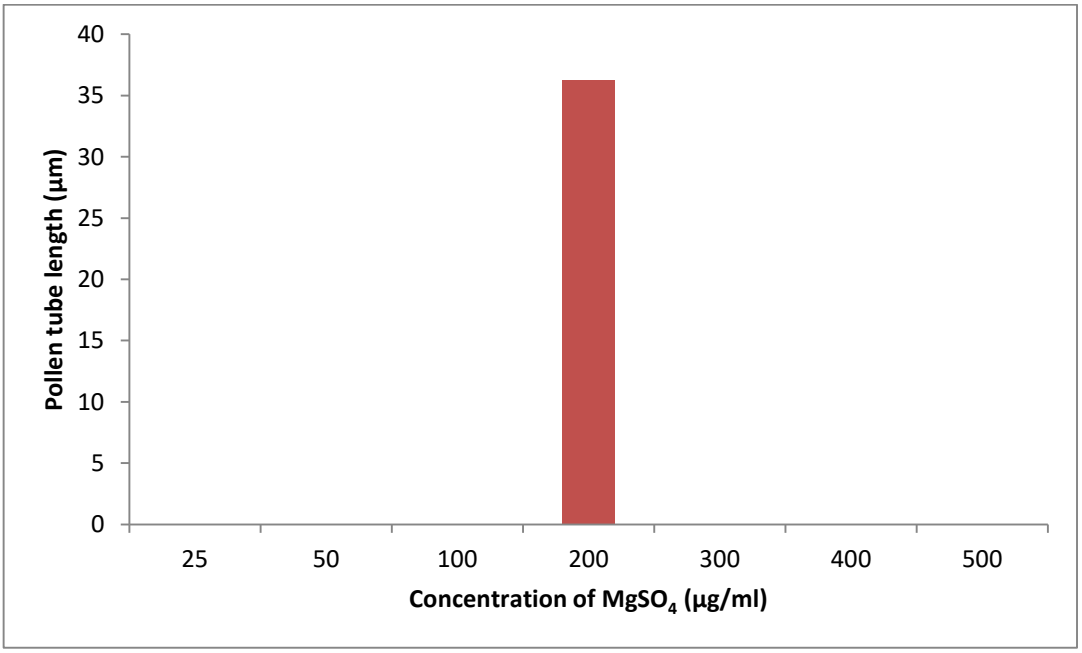
Table 36. *Floscopa scandens*: In-vitro pollen germination - effect of magnesium sulphate

Sl. No.	Concentration of $MgSO_4$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	-	-
4	200	5.52±2.23	36.24±5.25
5	300	-	-
6	400	-	-
7	500	-	-

Graph 41. *Floscopa scandens*: Effect of magnesium sulphate - pollen germination



Graph 42. *Floscopa scandens*: Effect of magnesium sulphate - pollen tube length



5.3.3 Stigma biology

5.3.3.1 Stigma morphology

The stigma is of dry type and the stigmatic surface measures about 73.047±0.49 μm and the entire surface is covered by papillae of about 38.13±1.33 μm. The style is solid.

5.3.3.2 Stigma receptivity

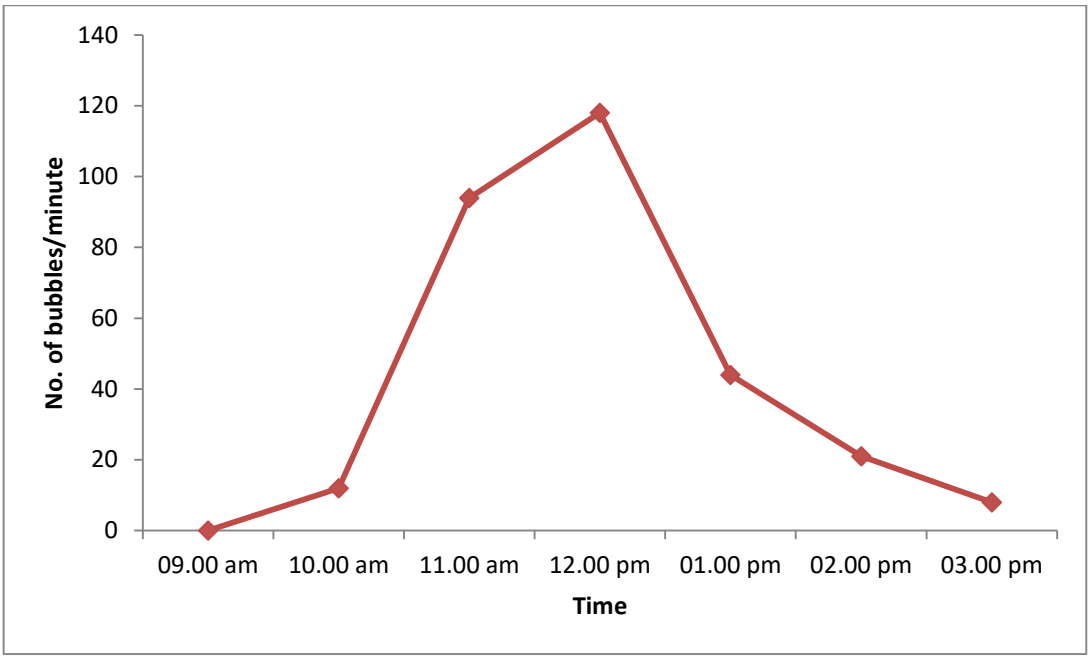
5.3.3.2.1 Stigma receptivity- hydrogen peroxide test

Stigmatic surface showed maximum receptivity around 11.00 am–12.00 pm in hydrogen peroxide test (Fig. 37 C & D; table 37; graph 43).

Table 37. *Floscopa scandens*: Stigma receptivity - hydrogen peroxide test

Sl. No.	Time of observation	No. of bubbles/minute ± S.E.
1	09.00 am	-
2	10.00 am	12±4
3	11.00 am	94±8
4	12.00 pm	118±4
5	01.00 pm	44±3
6	02.00 pm	21±1
7	03.00 pm	8±2

Graph 43. *Floscopa scandens*: Stigma receptivity - hydrogen peroxide test



5.3.3.2.2 Cytochemical localization of stigma-surface esterases

Cytochemical localization of stigma-surface esterases using α-naphthyl acetate demonstrated that the stigmatic surface was most receptive during a period between 11.00 am–12.00 pm (Fig. 37 A & B).

5.3.4 Pollination

5.3.4.1 Mode of pollination

Mode of pollination is entomophilous and autogamous.

5.3.4.2 Role of wind in pollination

Examination of the microscopic slides under revealed an absence of pollen grains thus ruling out wind pollination for the species.

5.3.4.3 Floral visitors and their behaviours

Insect visits started 10–20 minutes after anthesis. Insect visits were most frequent on sunny days as compared to humid cloudy days.

The flowers were visited by (Fig. 38–45) *Apis dorsata* (Apidae), *Apis florea* (Apidae), *Halictus* sp. (Halictidae), *Amegilla zonata* (Apidae), *Tetragonula iridipennis* (Apidae), *Ceratina* sp. (Apidae), and flies form the family Syrphidae. Of these, *Apis dorsata*, *Halictus* sp. 6 and *Amegilla zonata* exhibited high frequency of visits. They would hover above the flowers, working on the stamens, sometimes bundling up the stamens and stigma together. *Ceratina* sp. and *T. iridipennis* lands on the stamen and moves around within the flower whereas the syrphid flies, hangs on to the stamen and work on them. The orientation of the flowers and the arrangement of the stamens into upper and lower sets seem to ensure that while the insects handles the upper set of stamens their ventral abdomen gets dusted with pollen from the lower set of stamens. However this is not as much effective with *Ceratina* sp. and *T. iridipennis* as these insects works within the flower, going to and fro from the stamens, they get dusted on both halves of their body. More details on the floral visitors are given in the table 38.

Table 38. *Floscopa scandens*: Floral visitors and their behaviour

Sl.No.	Name of the taxa with family	Nature	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality		
							Field 1	Field 2	CU campus
1	*Apis dorsata* Apidae	Pollinator	11.00 am-01.30 pm	10-30 seconds	+++	High	✓	✓	✓
2	*Halictus* sp. 6 Halictidae	Pollinator	10.00 am-02.00 pm	10-30 seconds	+++	High	✓	✓	✓
3	*Amegilla zonata* Apidae	Pollinator	11.00 am-01.00 pm	3-5 seconds	+++	High	✓	✓	-
4	*Apis florea* Apidae	Pollinator	11.30 am-01.30 pm	5-20 seconds	++	Intermediate	✓	✓	-
5	*Tetragonula iridipennis*	Pollinator	12.00 pm-01.30 pm	10-25 seconds	++	Intermediate	✓	✓	✓
6	*Halictus* sp. 1 Halictidae	Pollinator	12.00 pm-01.00 pm	10-20 secconds	++	Intermediate	✓	-	✓
7	*Ceratina* sp. 3 Apidae	Pollinator	11.30 pm-01.30 pm	10-25 seconds	++	Intermediate	✓	✓	✓

Sl.No.	Name of the taxa with family	Nature	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality		
							Field 1	Field 2	CU campus
8	*Halictus* sp. 7 Halictidae	Pollinator	11.30 am-01.30 pm	10-20 seconds	++	Low	✓	-	-
9	Unidentified sp. 5 Syrphidae	Pollinator	12.00 pm-01.00 pm	10-25 seconds	++	Low	✓	-	✓
10	Unidentified sp. 7 Syrphidae	Pollinator	11.00 am-02.00 pm	10-25 seconds	++	Low	✓	-	-
11	Unidentified sp. 8 Syrphidae	Pollen robber	10.30 am-11.00 am	10-20 seconds	++	Low	✓	-	-

Stigma touch: +++ very good; ++ good; + poor

Frequency of visits: High (5–30 visits/day); Intermediate (1–5 visits/day); Low (<1 visit/day).

5.3.4.2 Pollination efficiency

Stigmas were observed under a microscope after the flowers closed. Stigmas of 48% of flowers showed the presence of pollen.

To study the pollination efficiency of individual visitors, stigmas were observed right after the first visit. It was found that *Apis dorsata* (Fig. 39), *Halictus* sp. 6 (Fig. 40) and *Amegilla zonata* (Fig. 41) were the most efficient pollinator of *F. scandens*.

5.3.5 Breeding system

The fruit set and seed set percentages from each of the pollination treatments are shown in the tables 39 & 40 and graphs 44 & 45.

5.3.5.1 Apomixis

No fruit set was observed in flowers bagged after emasculation, prior to anthesis.

5.3.5.2 Autogamy

Fruit set in flowers bagged before anthesis was 28.00% and 16.00% at population 1 and population 2 respectively.

5.3.5.3 Manual self-pollination (MSP)

Pollinating flowers by pollen from the same flowers showed 72.00% of fruit set in population 1 and 68.00% in population 2.

5.3.5.4 Manual cross-pollination (MCP)

Flowers pollinated by pollen from other plants showed 80.00% of fruit set in population 1 and 88.00% of fruit set in population 2.

5.3.5.5 Open pollination (OP)

Flowers pollinated by pollinators under natural conditions showed 76.00% of fruit set in population 1 and 68.00% in population 2.

Table 39. *Floscopa scandens*: Breeding system, Population 1

Sl. No.	Treatments	POP 1 Adivaram					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seed set
1	Apomixis	25	0	0	0	3	0
2	Autogamy	25	7	28.00	14	3	18.67
3	MSP	25	18	72.00	36	3	48.00
4	MCP	25	20	80.00	66	3	53.33
5	OP	25	19	76.00	38	3	50.67

Graph 44. *Floscopa scandens*: Breeding system - fruit set and seed set at population 1

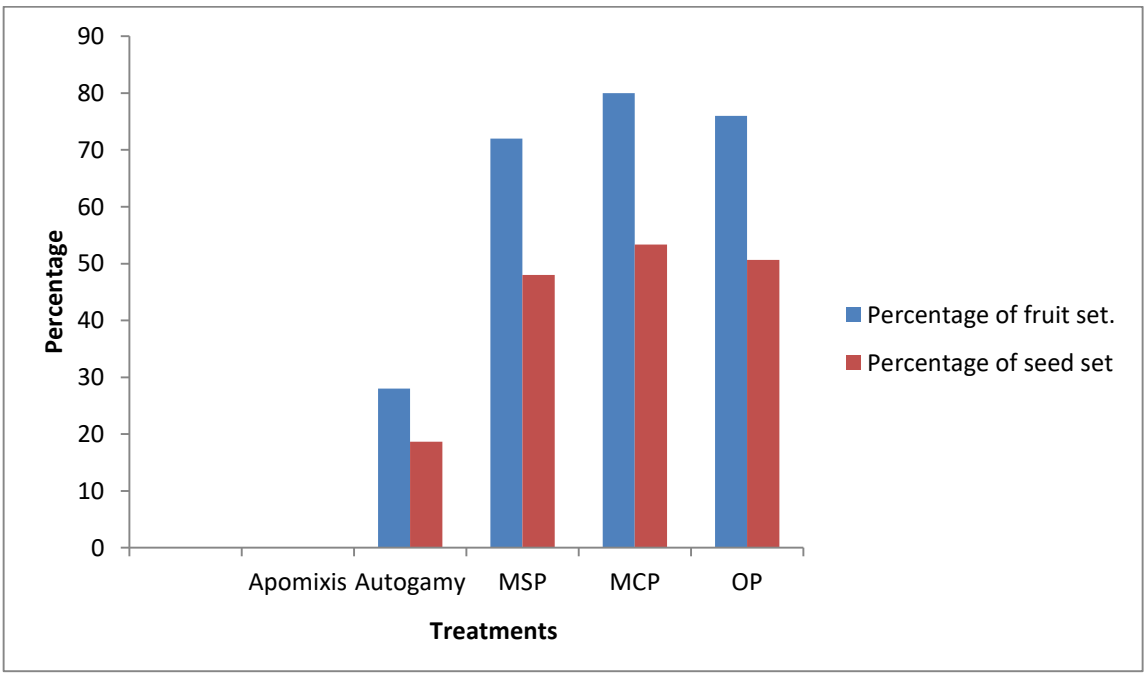
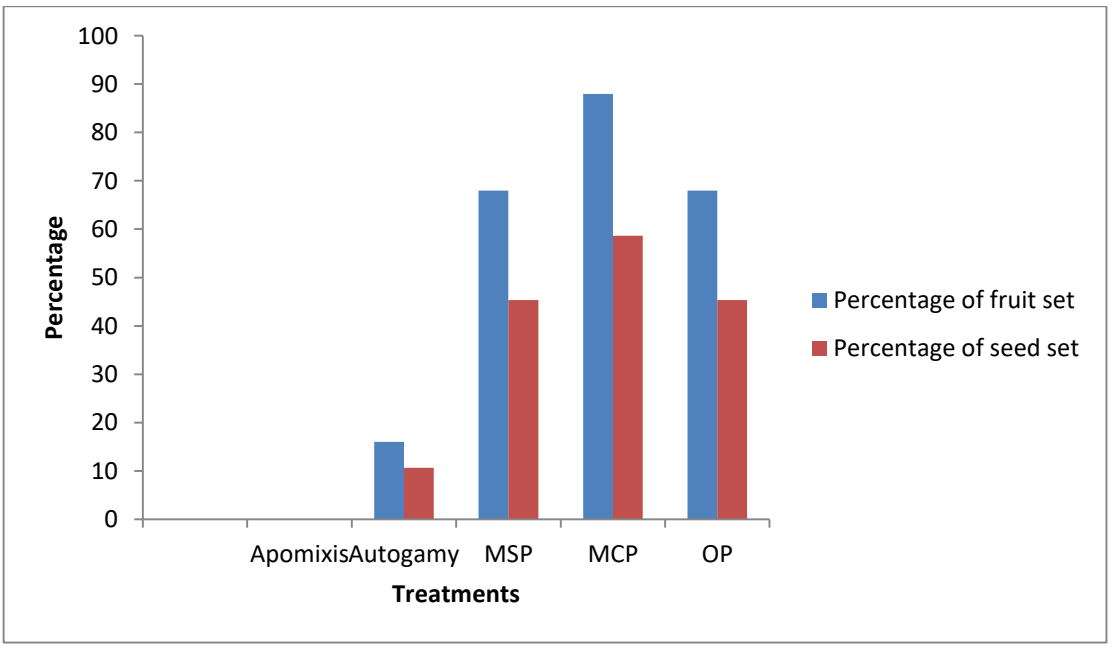


Table 40. *Floscopa scandens*: Breeding system, Population 2

Sl. No.	Treatments	POP 2 Kakkyam					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seed set
1	Apomixis	25	0	0	0	3	0
2	Autogamy	25	4	16.00	8	3	10.67
3	MSP	25	17	68.00	34	3	45.33
4	MCP	25	22	88.00	44	3	58.67
5	OP	25	17	68.00	34	3	45.33

Graph 45. *Floscopa scandens*: Breeding system - fruit set and seed set at population 2



5.3.6 Fruit and seed biology

After fertilization, the petals deliquesce and the sepals persist on the fruit. The fruits matured 4–5 days after fertilization and the capsules dehisced 7–9 days after fertilization (Fig. 35 H). Capsules are broadly ovoid, compressed, glabrous, bilocular with a single seed in each locule. Capsules dehisce along the septa, propelling the seeds out.

Seeds 2, greyish with a tinge of purple, hemispherical, dorsiventrally compressed with 12–16 radial ribs. Embryotega is central on the dorsal surface and hilum curved on the ventral surface. Seed surface are covered with polygonate cells with depressed edges and smooth projecting faces.

5.3.6.1 Flower-fruit ratio and ovule-seed ratio

Under natural conditions, the flower to fruit set ratio was 25:18 and the ovule to seed set ratio was 25:12

5.3.6.2 Seed germination

Mature seeds kept in a petri-dish over cotton soaked in water showed no germination whereas seeds sowed in pots under nursery condition showed 17% germination (Fig. 46).

Table 41. *Floscopa scandens*: Summary of floral characters

Sl. No.	Floral characters	Observations		
1	Flowering period	December to March		
2	Flower type	Zygomorphic, bisexual		
3	Flower colour	Puple-lilac		
4	Odour	Absent		
5	Nectar	Absent		
6	Anthesis time	10.20 am-10.40 am		
7	Anther dehiscence time	10.25 am-10.50am		
8	Anther dehiscence	Longitudinal		
9	No. of anthers/flower	6		
10	No. of staminodes/flower	0		
11	Mean no. of pollen grains/ anther	611±81 (LS) 308±66 (US)		
12	Mean no. of pollen grains/ flower	2757		
13	Mean no. of ovules/flower	3		
14	Pollen-ovule ratio	919:1		
15	Pollen structure	Spinulose and monolete		
16	Pollen size		P	E
		LS	31.31±0.70µm	21.26±0.5µm
		US	36.18±0.35µm	21.45±0.33µm
17	Pollen shape	Prolate		
18	Stigma type	Dry, papillate		
19	Pollen viability (max%)	94.26±0.63 (LS) 92.93±0.96 (US)		
20	Fruit type	Capsule		
21	Flower-fruit ratio	25:18		
22	Ovule-seed ratio	25:12		
23	Flower closing time	02.30 pm		

5.4 *Murdannia nudiflora* (L.) Brenan

Murdannia nudiflora, commonly called 'dove weed' (Fig. 2 D; Fig. 47 A & B), is reported to be invasive (Faden, 2000; GCW, 2007) in most parts of the United States, Central and South Americas, and Western Australia (Keighery, 1982; Waterhouse, 1993; Faden, 2000).

Annuals with partly decumbent stems. The stems root at their nodes when in touch with the soil. Internodes with a line of cilia. Basal leaves of the main axis forms a rosette; sheath 0.5–1 cm long, either glabrous or ciliate, with cilia spread across the surface or confined to a line along the fused margins; lamina 3–10 × 0.4–0.9 cm, alternate, mostly glabrous but sometimes minutely hairy. Inflorescence terminal and axillary, consisting of single or sometimes paired pedunculate (0.7–13 mm) cincinni. Flowers bisexual (rarely male), trimerous. Sepals 2–2.5 × 1.5 mm, free, ovate–elliptic, pale green, glabrous. Petals 5–7 × 5–6 mm, free, obovate, lilac to lavender. There are two dorsifixed antisepalous stamens with dense hairs at the basal half of the filament. Anther lobes deep bluish purple with purplish-white connectives, and dehisce longitudinally to release ellipsoid, creamy-yellowish white pollen. There are four staminodes, of which three are antipetalous and one is antisepalous. They are either glabrous or possess a few hairs at the base with trilobed, white antherodes; the antherode of the antisepalous staminode is reduced to a knob. The ovary is greenish and glabrous, while the style is white, with papillate stigma. Capsules are ellipsoid–triangular, with two uniseriate seeds in each locule (Fig. 48 A-G).

5.4.1 Phenology

5.4.1.1 Population and seasonal phenology

Seedlings began to appear by late May to the beginning of June, with the onset of monsoon. Their life cycle lasts about 4–6 months and the population is completely wiped out by November to December.

Flowering began by the end of June to the beginning of July and peak flowering was observed during the end of July to the beginning of October.

5.4.1.2 Flowering phenology

An inflorescence flowered 8–14 days after initiation. Within a cincinnus flowering occurs in acropetal succession and each inflorescence would produce about 3–12 flowers (Fig. 49 A-H).

5.4.1.3 Intra-floral phenology

Anthesis usually took place between 10.30 to 11.30 am, but in shaded areas it was 10–15 minutes late. The species also produced cleistogamous flowers. Anthers dehisce within 5–8 minutes of anthesis (Table 53). By approximately 2.00–2.30 pm, all flowers were closed and further deliquesced (Fig. 50 A-H).

5.4.2 Pollen biology

5.4.2.1 Pollen morphology

Pollen grains are prolate, spinulose and monolete (Fig. 51 A & B). The polar (P) and equatorial (E) axes measured 51.82±0.81 μm and 24.75±0.38 μm respectively (n=50).

5.4.2.2 Pollen biochemical analysis

The pollen grains stained with I_2KI solution became brownish black, indicating the presence of starch and those stained with Sudan black became

black indicating the presence of lipid. On staining with Coomassie Brilliant Blue, the pollen grains became blue tinged indicating the presence of protein (Fig. 51 E-H).

5.4.2.3 Pollen production

An anther produced 799 ± 41 (mean ± S.E.) pollen on average.

5.4.2.4 Pollen-ovule ratio

As each flower bears two anthers, a flower on average produces about 1598 pollen grains and the pollen to ovule ratio of a flower was 533:2.

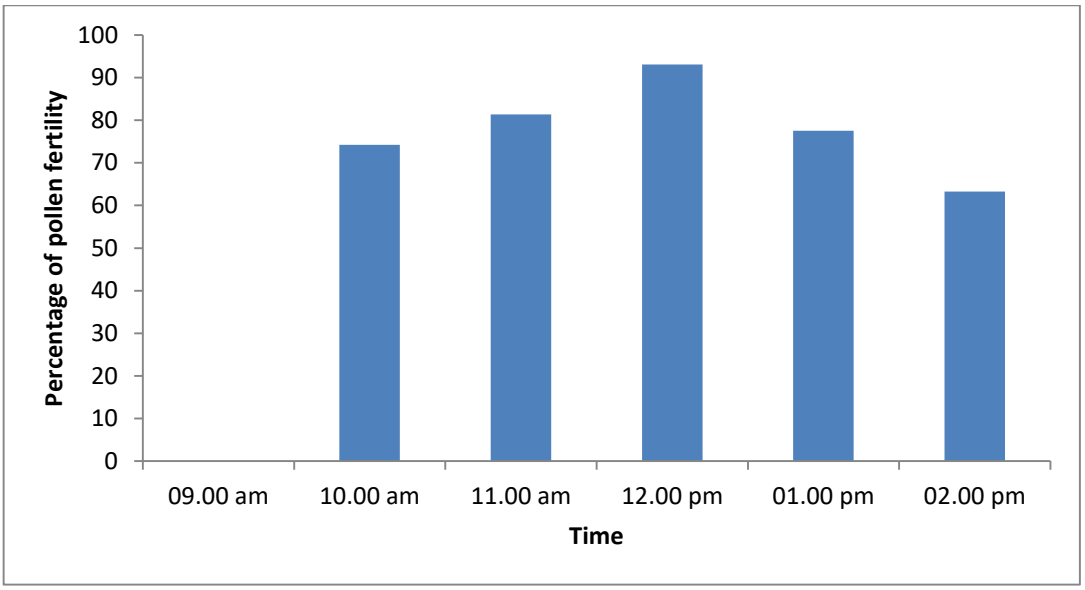
5.4.2.5 Pollen fertility and sterility

Acetocarmine staining technique showed (Fig. 51 D; table 42; graph 46) maximum fertility at around 11.00 am–12.00 pm (93.06±2.72%).

Table 42. *Murdannia nudiflora*: Pollen fertility - acetocarmine test

Sl. No.	Time of observation	Percentage of pollen fertility ±S.E
1	09.00 am	-
2	10.00 am	74.21±0.95
3	11.00 am	81.33±1.12
4	12.00 pm	93.06±2.72
5	01.00 pm	77.56±0.36
6	02.00 pm	63.22±1.82

Graph 46. *Murdannia nudiflora*: Pollen fertility - acetocarmine test



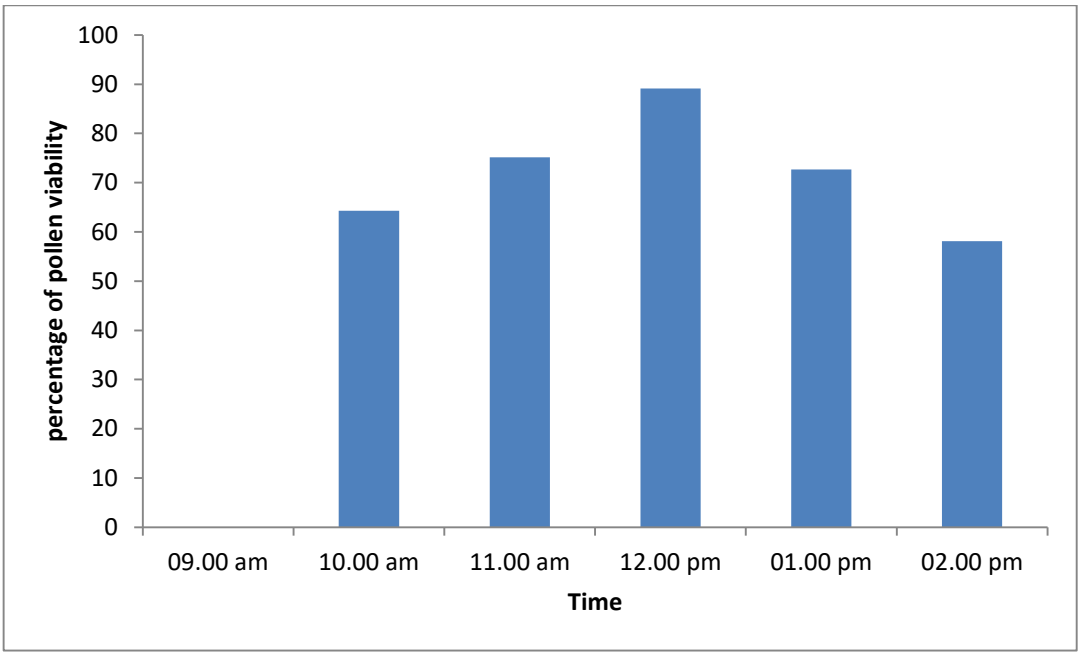
5.4.2.6 Pollen viability

Pollen became viable approximately 30 minutes before anthesis (Fig. 51 C; table 43; graph 47). Pollen viability was at its maximum around a period of 11.00 am to 12.00 pm (89.12±0.84), after which it declined.

Table 43. *Murdannia nudiflora*: Pollen viability - tetrazolium test

Sl. No.	Time of observation	Percentage of pollen viability±S.E
1	09.00 am	-
2	10.00 am	64.32±0.85
3	11.00 am	75.12±0.62
4	12.00 pm	89.12±0.84
5	01.00 pm	72.66±1.36
6	02.00 pm	58.12±1.6

Graph 47. *Murdannia nudiflora*: Pollen viability - tetrazolium test



5.4.2.7 Effect of organic and inorganic nutrients on in-vitro pollen germination

In-vitro pollen germination studies (Fig. 51 E; table 44; graphs 48 & 49) showed that maximum germination percentage (49.36±0.12%) and maximum pollen tube length (98.32±5.8 µm) was observed in Brewbaker & Kwack's medium. Sucrose solution of 10% gave the next best result with 20.36±30 % germination and 34.27±3.6 µm pollen tube length.

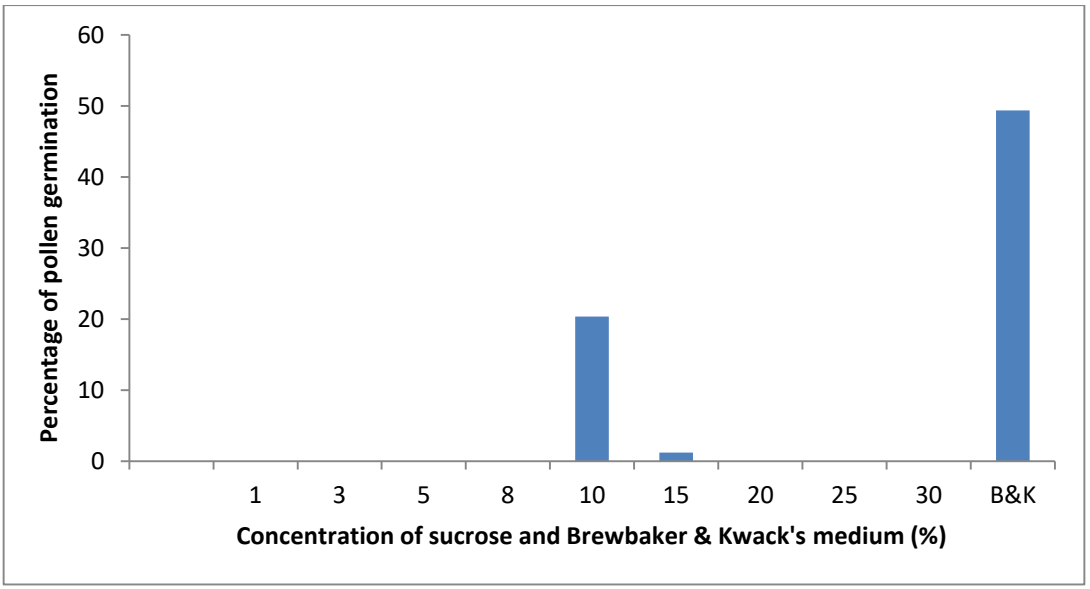
Amongst calcium nitrate solutions (Table 45; graphs 50 & 51) of different concentrations, *M. nudiflora* pollen grains showed germination in 200 µg/ml solution only (08.52±1.34% and 60.32±4.82 µm). 100 µg/ml solution of pottassium nitrate (Table 46; graphs 52 & 53) gave the maximum percentage of pollen germination (12.01±0.23%) as well as the maximum pollen tube length (29.12±2.4 µm) amongst solutions of different concentrations. Boric acid solution (Table 47; graphs 54 & 55) of 200 µg/ml gave the maximum

germination percentage (11.02±1.1%) as well as maximum pollen tube length (26.25±3.8 µm) whereas in magnesium sulphate solutions (Table 48; graphs 56 & 57), pollen grains germinated only at a concentration of 200 µg/ml (5.86±3.4% and 27.04±2.7).

Table 44. *Murdannia nudiflora:* In-vitro pollen germination in sucrose and Brewbaker & Kwack's medium.

Concentration (%)	**Pollen germination ± S.E. (%)**	**Pollen tube length ± S.E. (µm)**
Sucrose		
1	-	-
3	-	-
5	-	-
8	-	-
10	20.36±30	34.27±3.6
15	1.2±0.62	22.64±4.3
20	-	-
25	-	-
30	-	-
Brewbaker & Kwack's medium	49.36±0.12	98.32±5.8

Graph 48. *Murdannia nudiflora*: Effect of sucrose and Brewbaker and Kwack's medium - pollen germination



Graph 49. *Murdannia nudiflora*: Effect of sucrose and Brewbaker and Kwack's medium - pollen tube length

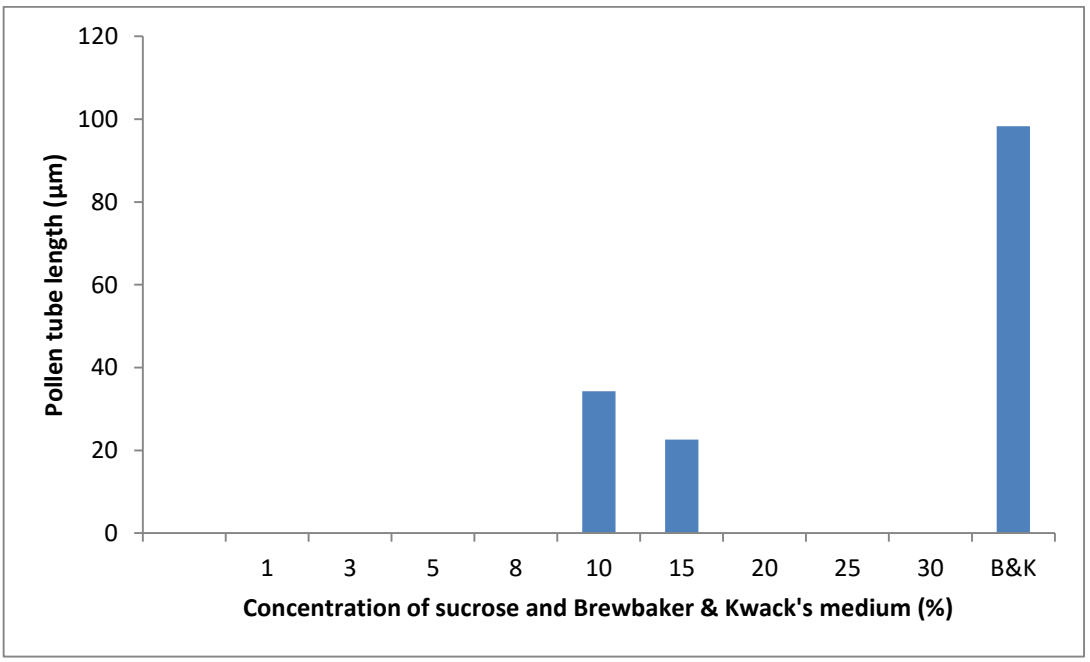
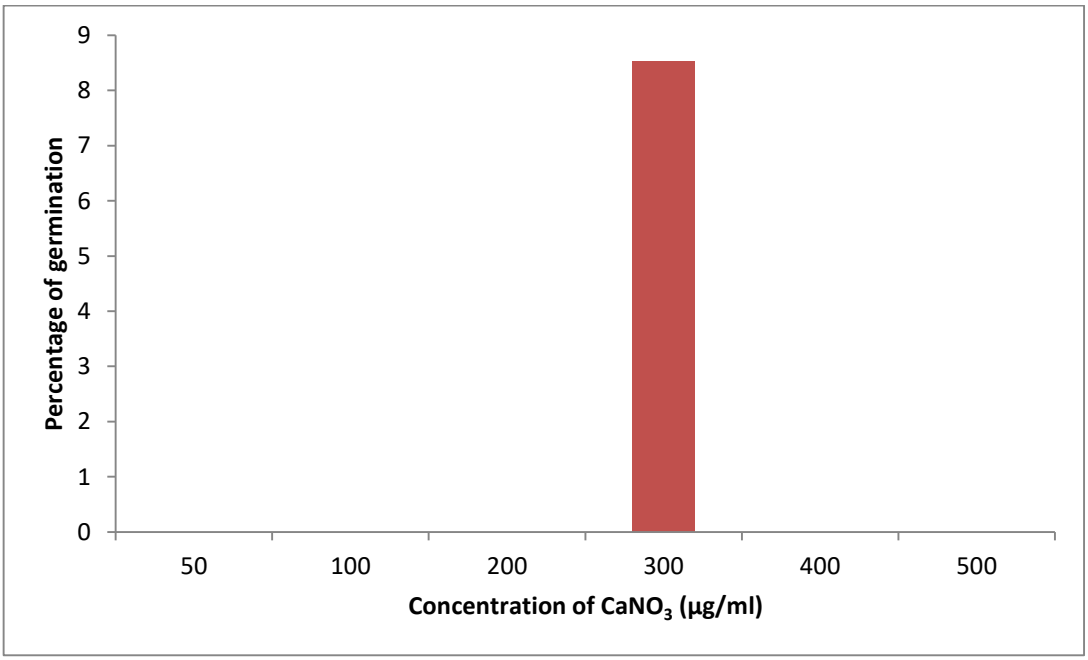


Table 45. *Murdannia nudiflora:* In-vitro pollen germination - effect of calcium nitrate

Sl. No.	Concentration of $CaNO_3$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	50	-	-
2	100	-	-
3	200	-	-
4	300	08.52±1.34	26±3.73
5	400	-	-
6	500	-	-

Graph 50. *Murdannia nudiflora*: Effect of calcium nitrate - pollen germination



Graph 51. *Murdannia nudiflora*: Effect of calcium nitrate - pollen tube length

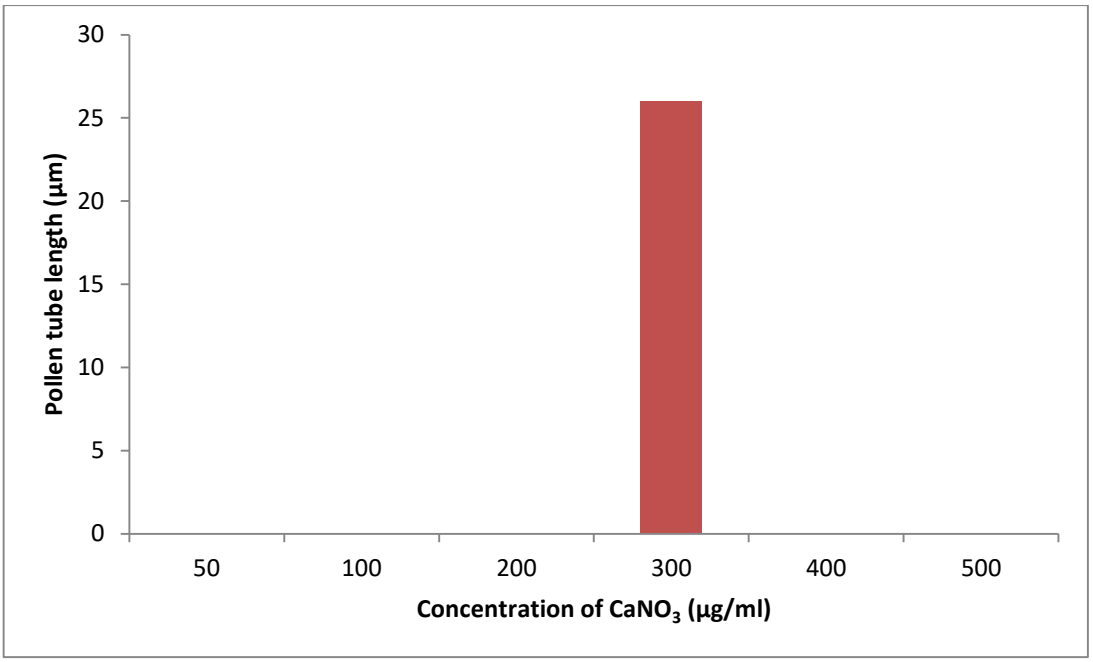
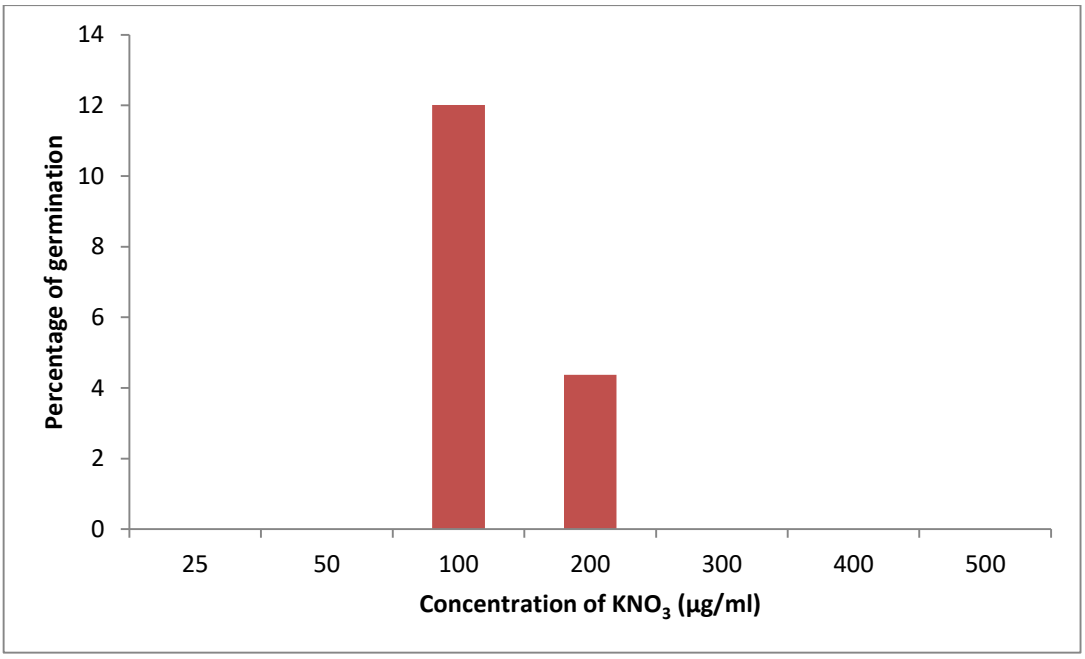


Table 46. *Murdannia nudiflora:* In-vitro pollen germination - effect of potassium nitrate

Sl. No.	Concentration of KNO_3 (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	12.01±0.23	29.12±2.4
4	200	4.37±2.8	22.20±1.2
5	300	-	-
6	400	-	-
7	500	-	-

Graph 52. *Murdannia nudiflora*: Effect of potassium nitrate - pollen germination



Graph 53. *Murdannia nudiflora*: Effect of potassium nitrate - pollen tube length

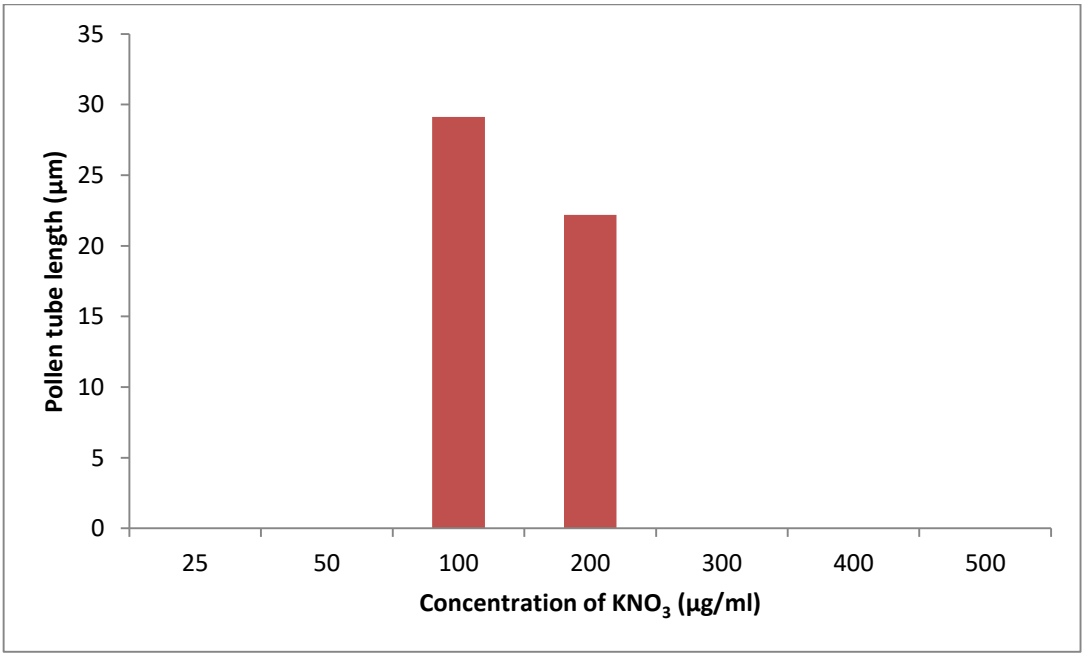
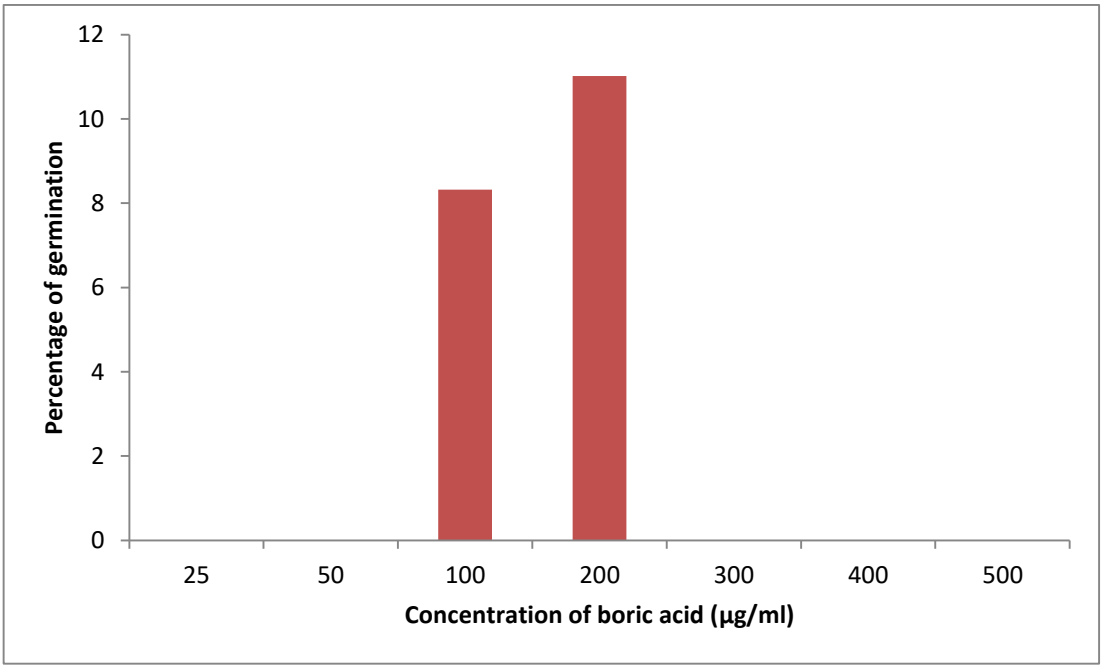


Table 47. *Murdannia nudiflora:* In-vitropollen germination - effect of boric acid

Sl. No.	Concentration of boric acid (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	08.32±2	26.19±2.6
4	200	11.02±1.1	26.25±3.8
5	300	-	-
6	400	-	-
7	500	-	-

Graph 54. *Murdannia nudiflora*: Effect of boric acid - pollen germination



Graph 55. *Murdannia nudiflora*: Effect of boric acid - pollen tube length

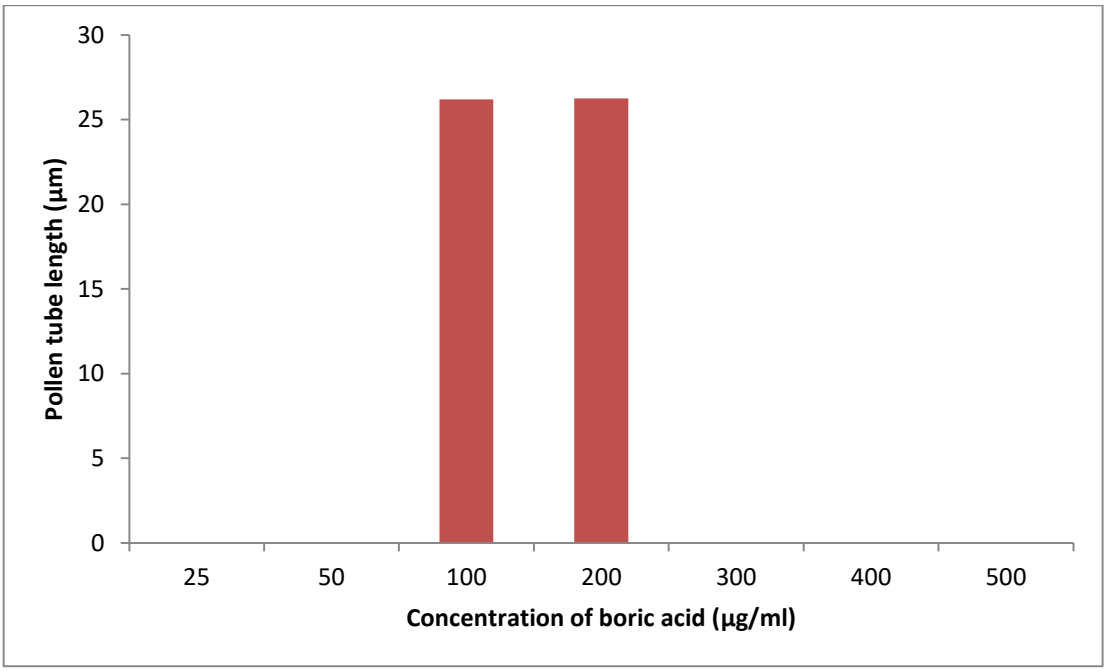
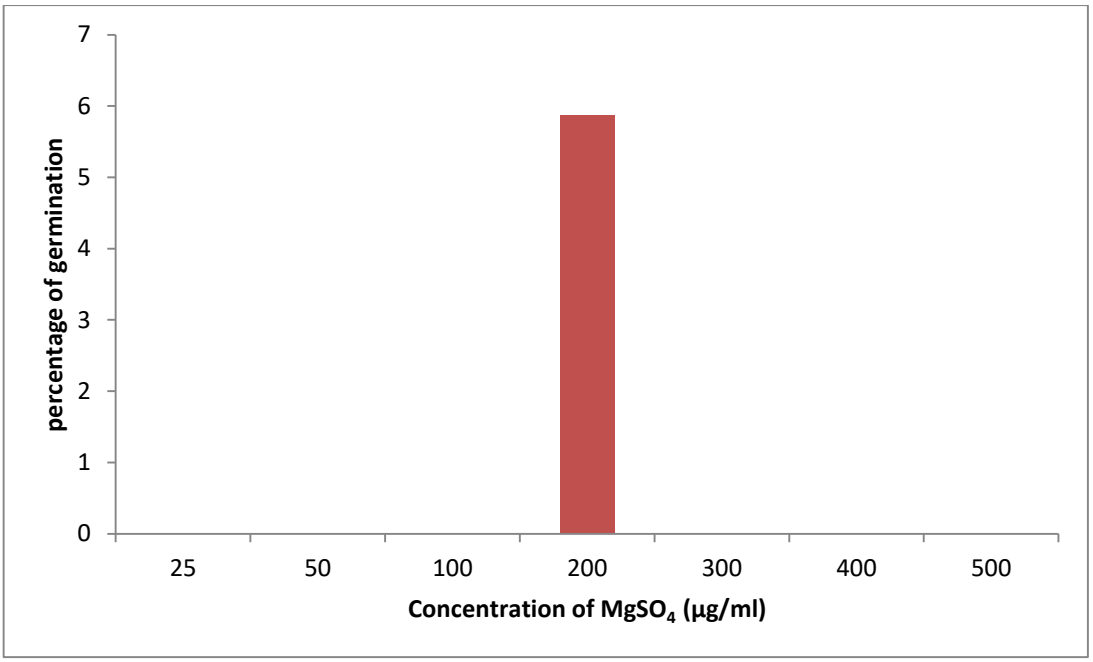


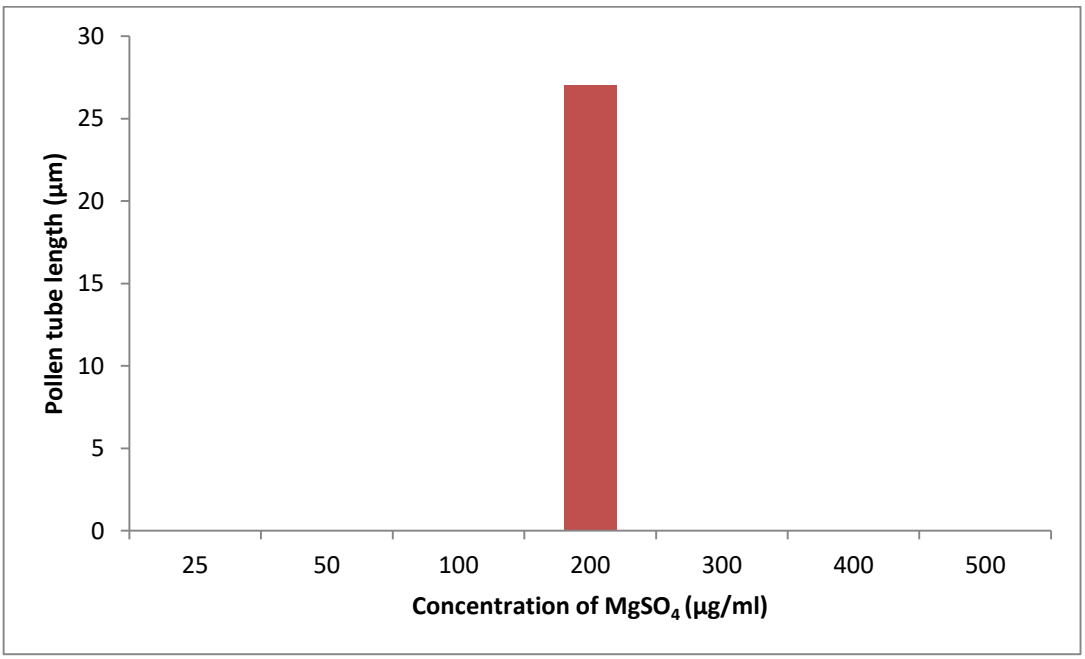
Table 48. *Murdannia nudiflora:* In-vitro pollen germination - effect of magnesium sulphate

Sl. No.	Concentration of $MgSO_4$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	-	-
4	200	5.86±3.4	27.04±2.7
5	300	-	-
6	400	-	-
7	500	-	-

Graph 56. *Murdannia nudiflora*: Effect of magnesium sulphate - pollen germination



Graph 57. *Murdannia nudiflora*: Effect of magnesium sulphate - pollen tube length



5.4.3 Stigma biology

5.4.3.1 Stigma morphology

The stigma is of dry type and the entire stigmatic surface measures about 114±1.22 µm. The surface is covered by papillae of about 62.12±1.3 µm. The style is solid.

5.4.3.2 Stigma receptivity

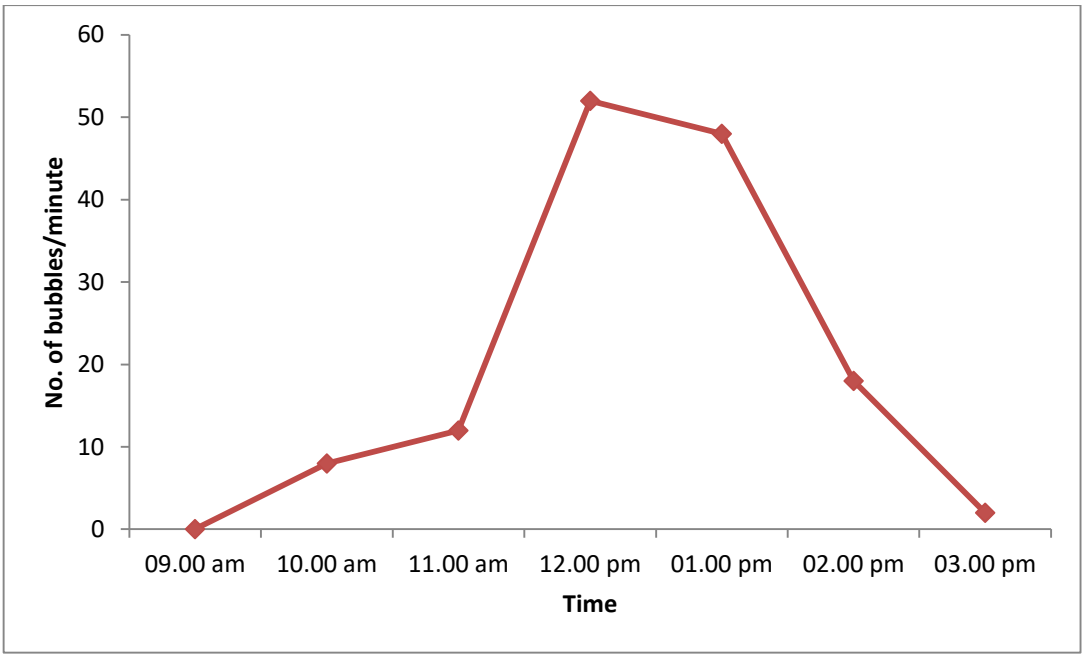
5.4.3.2.1 Stigma receptivity- hydrogen peroxide test

Stigmatic surface showed maximum receptivity around 12.00–01.00 pm in hydrogen peroxide test (Fig. 52 C & D; table 49; graph 58).

Table 49. *Murdannia nudiflora*: Stigma receptivity - hydrogen peroxide test

Sl. No.	Time of observation	No. of bubbles/minute ± S.E.
2	09.00 am	-
3	10.00 am	8±4
4	11.00 am	12±8
5	12.00 pm	52±6
6	01.00 pm	48±5
7	02.00 pm	18±6
8	03.00 pm	2±3

Graph 58. *Murdannia nudiflora*: Stigma receptivity - hydrogen peroxide test



5.4.3.2.2 Cytochemical localization of stigma-surface esterases

Cytochemical localization of stigma-surface esterases using α-naphthyl acetate demonstrated that the stigmatic surface was most receptive during a period between 12.00–01.00 pm (Fig. 52 A & B).

5.4.4 Pollination

5.4.4.1 Mode of pollination

Mode of pollination is entomophilous and autogamous.

5.4.4.2 Role of wind in pollination

Examination of the microscopic slides under a microscope revealed an absence of pollen grains thus ruling out wind pollination for the species.

5.4.4.3 Floral visitors and their behaviours

Ceratina sp. (Apidae), *Halictus* sp., *Osmia* sp. (Apidae), *Tetragonula iridipennis* (Apidae), unidentified species 9 (family Syrphidae), *Anthocoris* sp. (Anthochoridae) and *Aulacophora* sp. (Chrysomelidae) were observed (Fig. 53–58) visiting the flowers. Insect visits will begin even before anthesis and the insects will fly above the flowers looking for open flowers. *Ceratina* sp. will force its way inside buds that are beginning to open and work on them sometimes. *Osmia* sp., *T. iridipennis, Halictus* sp. and *Ceratina* sp. will land the stamens and move around within the flowers, working on the stamen while the unidentified species from the family Syrphidae will land on the stamen and work at them, staying on and repositioning itself on the stamens for the most part.

Anthocoris sp. and *Aulacophora* sp. were identified as predators and they eat the stamens, staminodes and even the petals.

Insect visits were limited or sometimes ceased on rainy days or on cloudy days with high humidity. More details on the floral visitors are given in the table 50.

Table 50. *Murdannia nudiflora*: Floral visitors and their behaviours

Sl. No.	Name of the taxa with family	Nature	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality	
							Field 1	CU campus
1	*Ceratina* sp.3 Apidae	Pollinator	10.30 am-01.30 pm	10-25 seconds	+++	High	✓	✓
2	*Osmia* sp. Apidae	Pollinator	10.30 am-01.30 pm	10-20 seconds	+++	High	✓	✓
3	Unidentified sp. 9 Syrphidae	Pollinator	11.00 am-02.00 pm	10-35 seconds	+++	Intermediate	-	✓
4	*Tetragonula iridipennis* Apidae	Pollinator	11.30 am-01.00 pm	5-25 seconds	+++	Intermediate	✓	✓
5	*Halictus* sp.2 Halictidae	Pollinator	11.30 pm-01.30 pm	10-20 seconds	+++	Low	-	✓
6	*Halictus* sp.8 Halictidae	Pollinator/	11.30 pm-01.00 pm	10-20 seconds	+++	Low	✓	-
7	*Anthocoris* sp. (Anthochoridae) bug	Predator	Random	-	-	-	-	✓
8	*Aulacophora* sp. (Chrysomelidae)	Predator	Random	-	-	-	-	✓

Stigma touch: +++ very good; ++ good; + poor
Frequency of visits: High (5–30 visits/day); Intermediate (1–5 visits/day); Low (<1 visit/day).

5.4.4.2 Pollination efficiency

Stigmas were observed under a microscope after the flowers closed. Stigmas of 68% of flowers showed the presence of pollen.

To study the pollination efficiency by individual visitors, stigmas were observed right after the first visit. It was found that *Ceratina* sp. 3 (Fig. A-F) is the most efficient pollinator of *M. nudiflora*.

5.4.5 Breeding system

The fruit set and seed set percentages from each of the pollination treatments are shown in the tables 51 & 52 and graphs 59 & 60.

5.4.5.1 Apomixis

No fruit set was observed in flowers bagged after emasculation, prior to anthesis.

5.4.5.2 Autogamy

Fruit set in flowers bagged before anthesis was 48.57% and 46.67% at population 1 and population 2 respectively.

5.4.5.3 Manual self-pollination (MSP)

Pollinating flowers by pollen from the same flowers showed 94.29% of fruit set in population 1 and 93.33% in population 2.

5.4.5.4 Manual cross-pollination (MCP)

Flowers pollinated by pollen from other plants showed 97.14% of fruit set in population 1 and 96.67% of fruit set in population 2.

5.4.5.5 Open pollination (OP)

Flowers left to be pollinated by natural pollinators under natural conditions showed 74.89% of fruit set in population 1 and 70.00% in population 2.

Table 51. *Murdannia nudiflora*: Breeding system, Population 1

Sl. No.	Treatments	POP 1 Vellimadukunnu					
		No. of flowers treated	No. of fruits developed	Percen-tage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seed set
1	Apomixis	35	0	0	0	6	0
2	Autogamy	35	17	48.57	98	6	46.67
3	MSP	35	33	94.29	168	6	80
4	MCP	35	34	97.14	173	6	82.38
5	OP	35	26	74.89	134	6	63.80

Graph 59. *Murdannia nudiflora*: Breeding system - fruit set and seed set at population 1

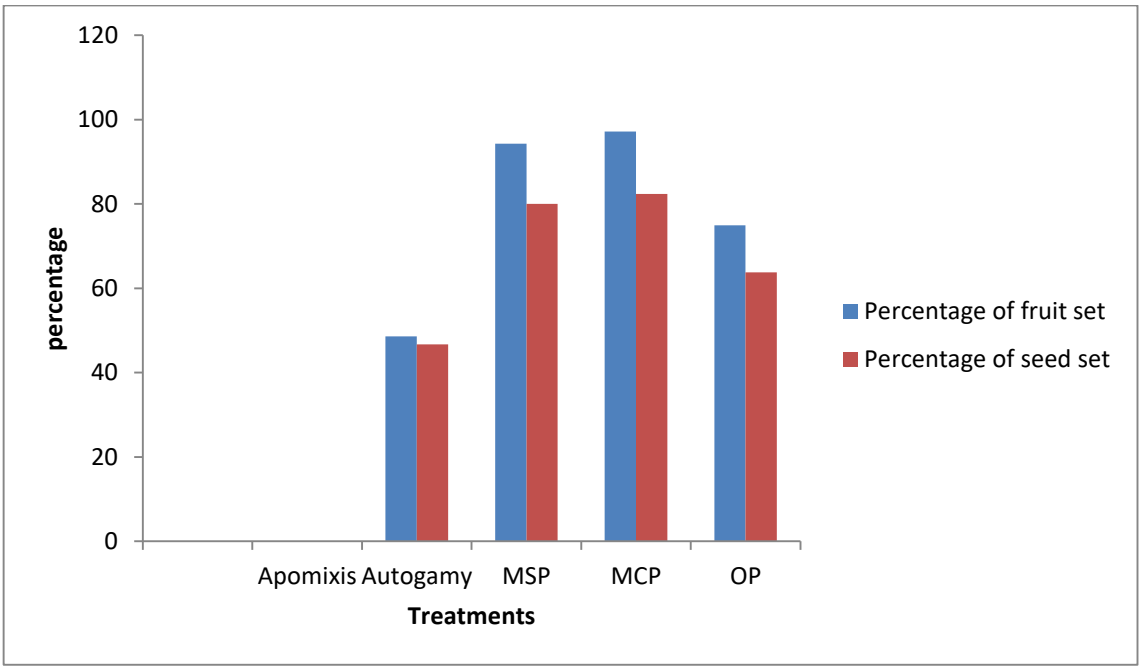
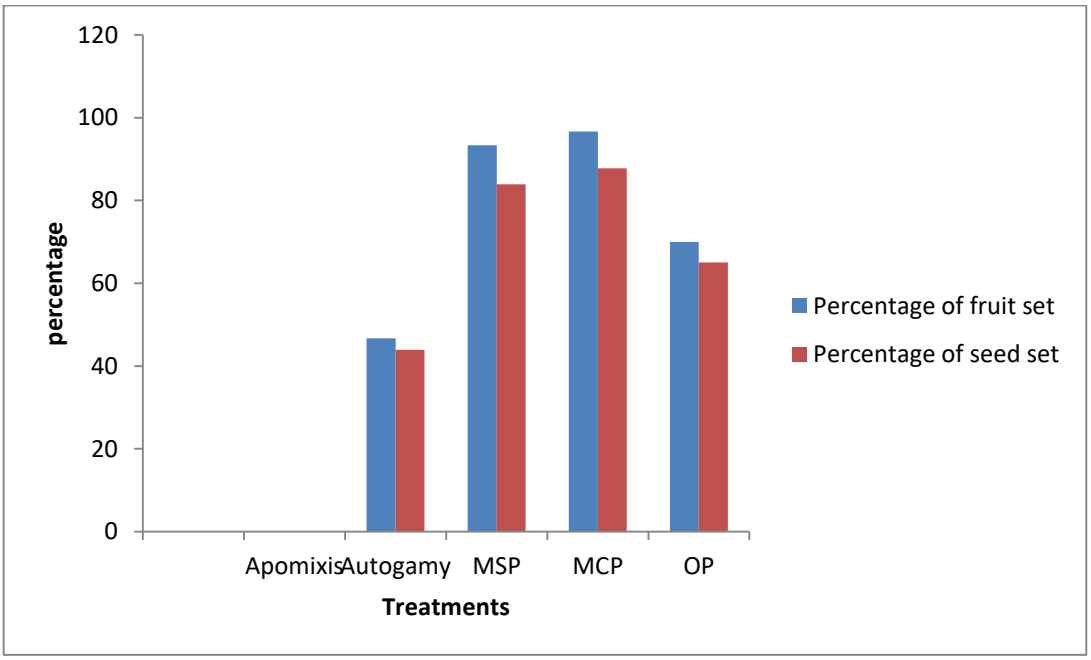


Table 52. *Murdannia nudiflora*: Breeding system, Population 2

Sl. No.	Treatments	POP 2 Vellimadukunnu					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seed set
1	Apomixis	30	0	0	0	6	0
2	Autogamy	30	14	46.67	79	6	43.89
3	MSP	30	28	93.33	151	6	83.89
4	MCP	30	29	96.67	158	6	87.78
5	OP	30	21	70.00	117	6	65.00

Graph 60. *Murdannia nudiflora*: Breeding system - fruit set and seed set at population 2



5.4.6 Fruit and seed biology

After fertilization the petals fall off from the flower and the sepal and style persists on the fruit. The fruits matured 3–4 days after fertilization and the capsules dehisced 6–8 days after fertilization. Capsules are ellipsoid–globose, slightly 3-faced, glabrous, trilocular, trivalved, locules two seeded. The capsules dehisce along the septa (Fig. 50 G & H).

Seeds 6, sometimes 5, brown, deltoid, base truncate, dorsiventral, dorsal surface convex and ventral carinate, embryotega lateral to semilateral, hilum punctiform. Testa ribbed, primary polygonate cells with deep depressions and secondary polygonate cells with elevated edges and flat faces.

5.4.6.1 Flower-fruit ratio and ovule-seed ratio

Under natural conditions, the flower to fruit set ratio was 65:47 and the ovule to seed set ratio was 390:251.

5.4.6.2 Seed germination

Mature seeds kept in a petri-dish over cotton soaked in water showed 12% germination whereas seeds sowed in pots under nursery condition showed 41% germination.

Murdannia nudiflora shows hypogeal germination pattern (Fig. 59).

Table 53. *Murdannia nudiflora*: Summary of floral characters

Sl. No.	Floral characters	Observations
1	Flowering period	July to October
2	Flower type	Zygomorphic, bisexual
3	Flower colour	Lilac-lavender
4	Odour	Absent
5	Nectar	Absent
6	Anthesis time	10.30-11.30 a m
7	Anther dehiscence time	10.35-11.3 am
8	Anther dehiscence	Longitudinal
9	No. of anthers/flower	2
10	No. of staminodes/flower	4
11	Mean no. of pollen grains/ anther	799±41
12	Mean no. of pollen grains/ flower	1598
13	Mean no. of ovules/flower	6
14	Pollen- ovule ratio	533:2
15	Pollen structure	Spinulose and monolete
16	Pollen size	51.82±0.81µm (P) 24.75±0.38 µm (E)
17	Pollen shape	Prolate
18	Stigma type	Dry, papillate
19	Pollen viability (max%)	89.12±0.84
20	Fruit type	Capsule
21	Flower-fruit ratio	65:47
22	Ovule-seed ratio	390:251
23	Flower closing time	02.00–02.30 p.m.

5.5 *Rhopalephora scaberrima* (Blume) Faden

Rhopalephora scaberrima, also known as the 'rough day flower' is a weedy herb, commonly found in the semi evergreen regions of the Indo-Malaysian areas (Fig. 2 E; Fig 60 A & B).

Erect to partly decumbent annual or perennial herbs, 1–1.5 m tall, branched, rooting at the lower nodes. Leaves distichous or spirally arranged; sheath 2.54 cm long, ciliated at the mouth; petiole 2 cm long; lamina 15–16.5 × 3–3.5 cm, oblong–lanceolate, acuminate at apex, hispid above, glabrous below. Inflorescence is a lax terminal and axillary, long pedunculate thyrse composed of largely bisexual, sometimes male, trimerous flowers. In a population, about13.74±1.5% of flowers that bloom each day, are functionally male with varying degrees of retardation to the gynoecium. Pedicels 1–1.5 cm long. Sepals green, 2 mm long, glabrous, persistent. Petals pale lilac, two lateral petals about 6–8 mm long and the medial petal about 4–6 mm long. Stamens 3; anther dimorphic, with two lateral stamens (L) bearing purple basifixed bithecous anthers and a medial stamen (M) with yellow anthers and a broad connective. Staminodes 2 (rarely 3). Anthers dehisce longitudinally along the length of the thecae. Ovary glabrous pubescent, densely covered with hooked glandular hairs, 3-celled with 1 ovule in each cell; style 5 mm long; stigma papillate. Capsules asymmetrically obovoid, densely hooked hairy. Seeds dorsiventrally compressed, testa grey, reticulate to foveolate. (Fig. 61 A-G).

5.5.1 Phenology

5.5.1.1 Population phenology

Rhopalephora scaberrima, propagates through vegetative and sexual means. It is more commonly found in forests, alongside streams or rivers. Observations during the study revealed a clear seasonal pattern of flowering in natural populations. The seedlings began to appear by June and flowering

initiates by late July. The flowering period extends till January. Peak in flowering was observed during August–November. Under greenhouse conditions the flowering period was extended to February–March.

5.5.1.2 Flowering phenology

The first flower of the inflorescence bloomed within 7–10 days of the inflorescence initiation. On average an inflorescence produced about 10–21 flowers. Flowers develop in acropetal succession within each cincinnus. Flower bud took 45 days from initiation, to full bloom (Fig. 62 A-H).

5.5.1.3 Intra-floral phenology

Anthesis began around 06.30–07.00 am. In a population, the flowers exposed to high intensity of light or even direct light, opened earlier than those in the shade. Anther dehisces 5-10 minutes after anthesis. Insects visiting before anther dehiscence roam around the flowers and manipulate the anthers. The flowers withered around 11.30 am–12.00 pm (Fig. 63 A-D).

5.5.2 Pollen biology

5.5.2.1 Pollen morphology

Morphologically the pollen grains produced by the two types of stamen showed no significant differences. All the grains were perprolate, spinulose and monolete (Fig. 64 A & B). Pollen produced by the lateral stamen (L) was 70.99±0.67 μm (P) and 33.62±0.29 μm (E), and those from the medial stamen (M) was 68.53±0.61 μm (P) and 29.30± 0.30 μm (E), along the polar and equatorial axes respectively (n=50).

5.5.2.2 Pollen biochemical analysis

The pollen grains stained with I_2KI solution became brownish black, indicating the presence of starch and those stained with Sudan black became

black indicating the presence of lipid. On staining with Coomassie Brilliant Blue, the pollen grains became blue tinged indicating the presence of protein (Fig. F-H).

5.5.2.3 Pollen production

The medial stamen produced significantly more pollen than the lateral ones ($p<0.05$). Mean number of pollen produced by the lateral stamen was calculated to be 1678±228 and that by the medial stamen was 2791±127.

5.5.2.4 Pollen-ovule ratio

Average pollen production per flower was estimated to be about 6148 pollen grains and each flower produce 3 ovules thus the pollen to ovule ratio is 6148:3.

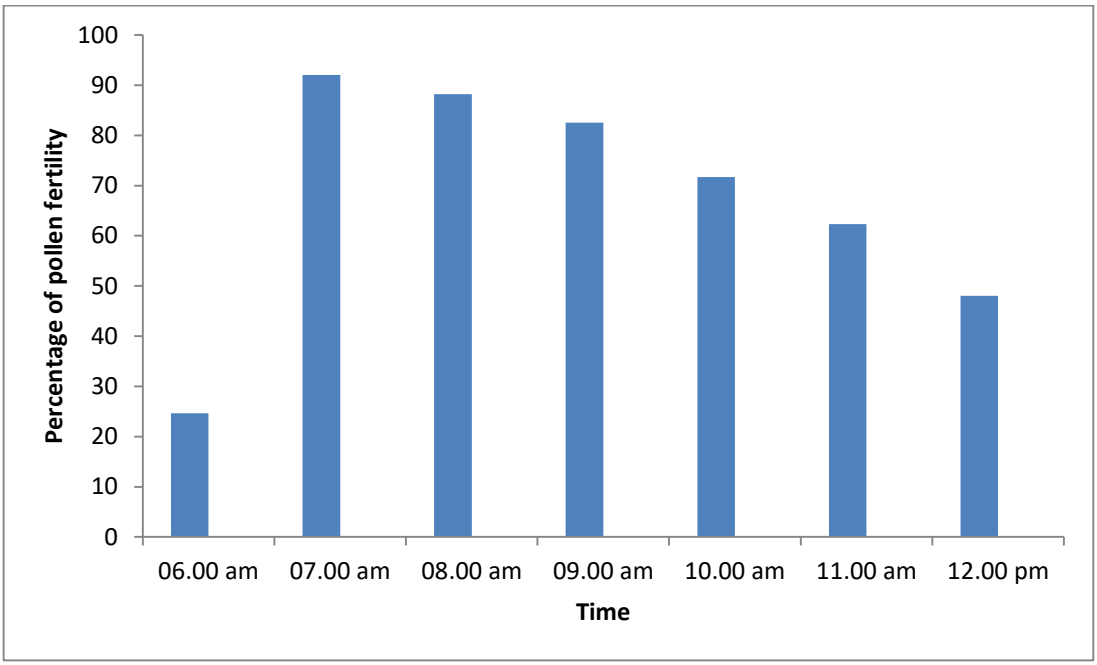
5.5.2.5 Pollen fertility and sterility

Acetocarmine staining technique showed maximum fertility (Fig. 64 D; table 54; graph 61) at 07.00–08.00 am (92.01±1.32%) in pollen from the lateral stamen, while the pollen from the medial stamen was infertile.

Table 54. *Rhopalephora scaberrima*: Pollen fertility - acetocarmine method

Sl. No.	Time of observation	Percentage of pollen fertility ±S.E	
		L	M
1	06.00 am	24.62±5.62	-
2	07.00 am	92.01±1.32	-
3	08.00 am	88.21±3.2	-
4	09.00 am	82.56±1.2	-
5	10.00 am	71.68±1.6	-
6	11.00 am	62.32±2.2	-
7	12.00 pm	48.03±3.1	-

Graph 61. *Rhopalephora scaberrima*: Pollen fertility - acetocarmine test



5.5.2.6 Pollen viability

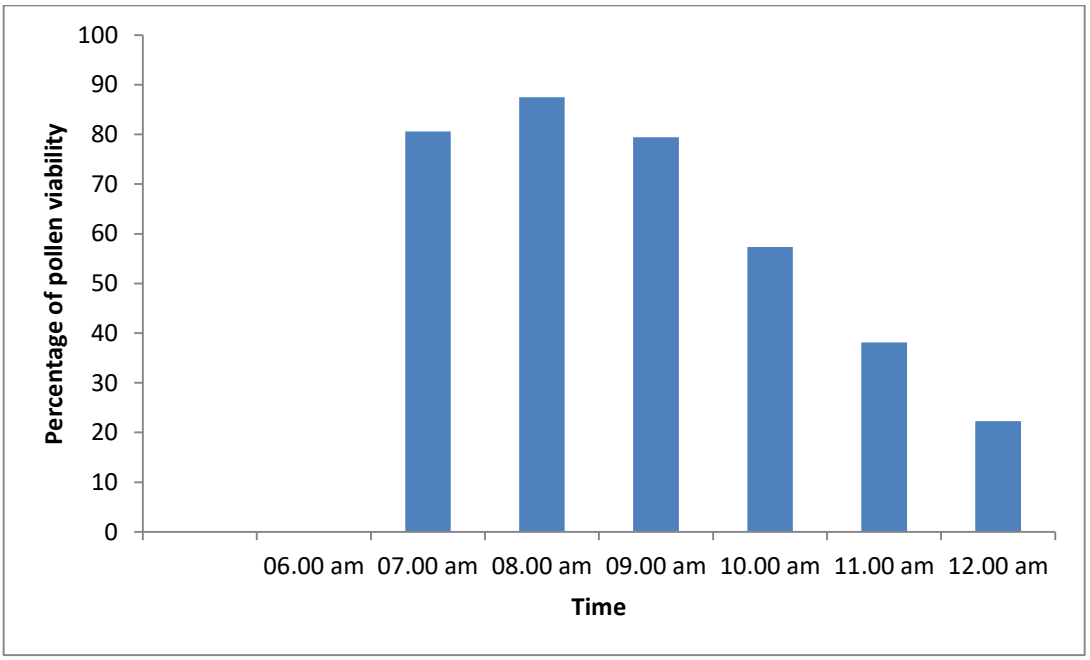
The stamens showed significant differences in their viability ($P<0.05$). While the lateral stamen produced viable pollen, the pollen produced by the medial stamen was non-viable as indicated by the lack of or partial staining (Fig. 64 C; table 55; graph 62).

Maximum viability was observed around 07.00–08.00 am (87.51±0.53) and then steadily decreased.

Table 55. *Rhopalephora scaberrima*: Pollen viability - tetrazolium method

Sl. No.	Time of observation	Percentage of pollen viability±S.E	
		L	M
1	06.00 am	-	-
2	07.00 am	80.58±0.22	-
3	08.00 am	87.51±4.53	-
4	09.00 am	79.43±2.1	-
5	10.00 am	57.31±0.41	-
6	11.00 am	38.12±0.6	-
7	12.00 pm	22.26±0.12	-

Graph 62. *Rhopalephora scaberrima*: Pollen viability - tetrazolium test



5.5.2.7 Effect of organic and inorganic nutrients on in-vitro pollen germination

In-vitro pollen germination studies showed that maximum germination (Fig. 64 E; table 56; graphs 63 & 64) percentage (86.84±2%) and maximum pollen tube length (132.36±3.28 µm) was observed in Brewbaker & Kwack's

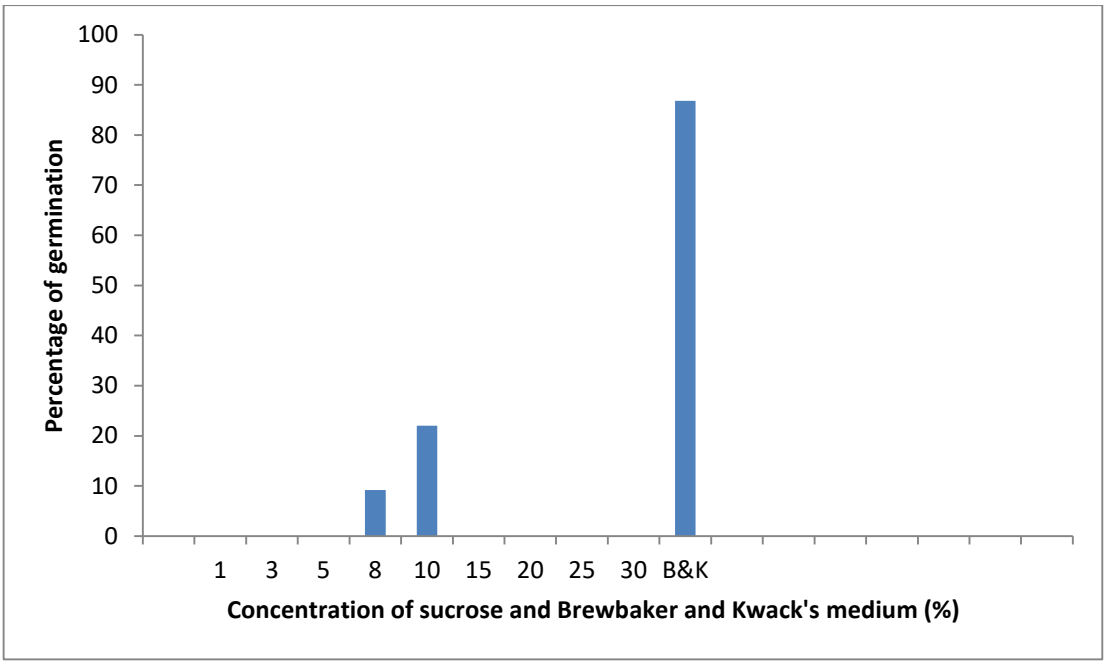
medium. Sucrose solution of 10% gave the next best result with 22.01±2.31% germination and 62.36±5.2 µm pollen tube length.

Amongst calcium nitrate solutions (Table 57; graphs 65 & 66) of different concentrations, *R. scaberrima* pollen grains showed maximum percentage of germination (13.72±1.09%) in 200 µg/ml and maximum pollen tube length was obtained in 400 µg/ml (60.32±4.82 µm). 200 µg/ml solution gave the maximum percentage of pollen germination (11.68±0.68%) as well as the maximum pollen tube length (62.02±3.8 µm) amongst potassium nitrate solutions (Table 58; graphs 67 & 68) of different concentrations. Boric acid solution (Table 59; graphs 69 & 70) of 100 µg/ml gave the maximum germination percentage (8.62 ±0.81%) as well as maximum pollen tube length (32.07±2.2 µm) whereas in magnesium sulphate solutions (Table 60; graphs 71 & 72), pollen grains germinated only at a concentration of 200 µg/ml (6.42±0.13% and 26.04±6.32 µm).

Table 56. *Rhopalephora scaberrima*: In-vitro pollen germination in sucrose and Kwack's medium.

Concentration (%) Sucrose	**Pollen germination ± S.E.(%)**	**Pollen tube length ± S.E. (µm)**
1	-	-
3	-	-
5	-	-
8	9.2±1.06	55±3.6
10	22.01±2.31	62.36±5.2
15	-	-
20	-	-
25	-	
30	-	-
Brewbaker & Kwack's medium	86.84±2.59	132.36±3.28

Graph 63. *Rhopalephora scaberrima*: Effect of sucrose and Brewbaker and Kwack's medium - pollen germination



Graph 64. *Rhopalephora scaberrima*: Effect of sucrose and Brewbaker and Kwack's medium - pollen tube length

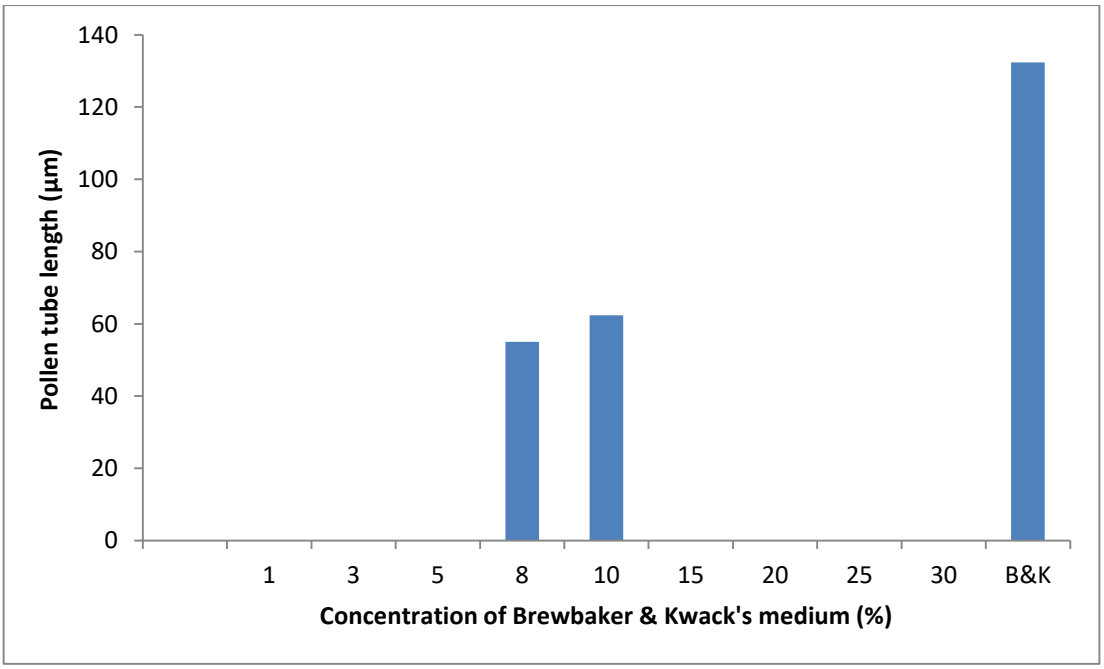
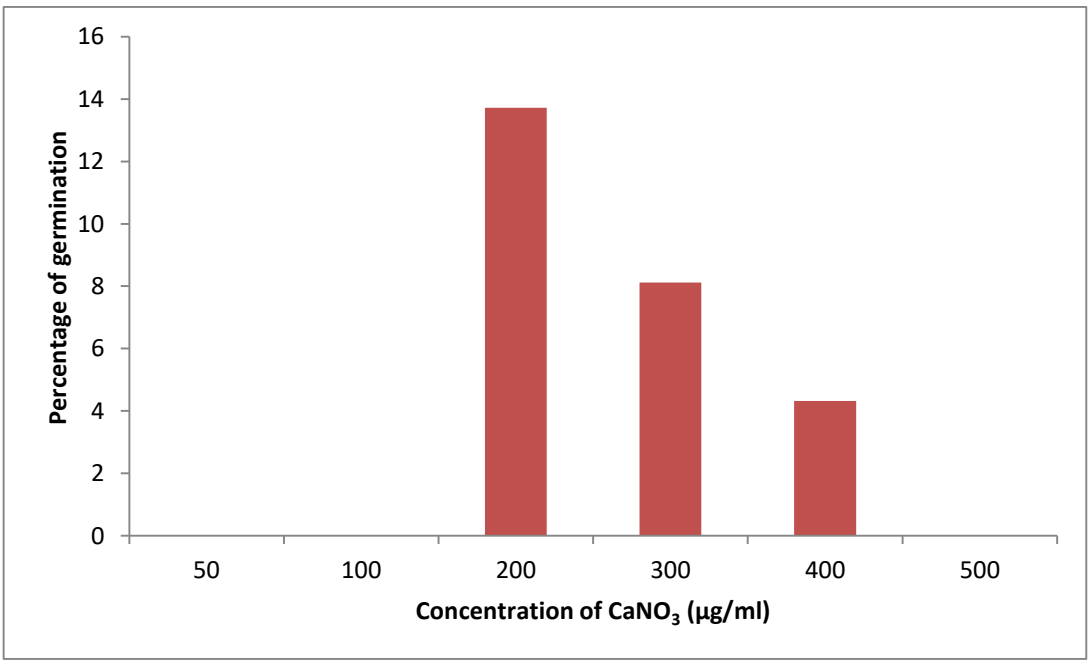


Table 57. *Rhopalephora scaberrima*: In-vitro pollen germination - effect of calcium nitrate

Sl. No.	Concentration of $CaNO_3$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	50	-	-
2	100	-	-
3	200	13.72±1.09	34.33±5.32
4	300	8.12±0.20	37.64±4.03
5	400	4.32±1.26	60.32±4.82
6	500	-	-

Graph 65. *Rhopalephora scaberrima*: Effect of calcium nitrate - pollen germination



Graph 66. *Rhopalephora scaberrima*: Effect of calcium nitrate - pollen tube length

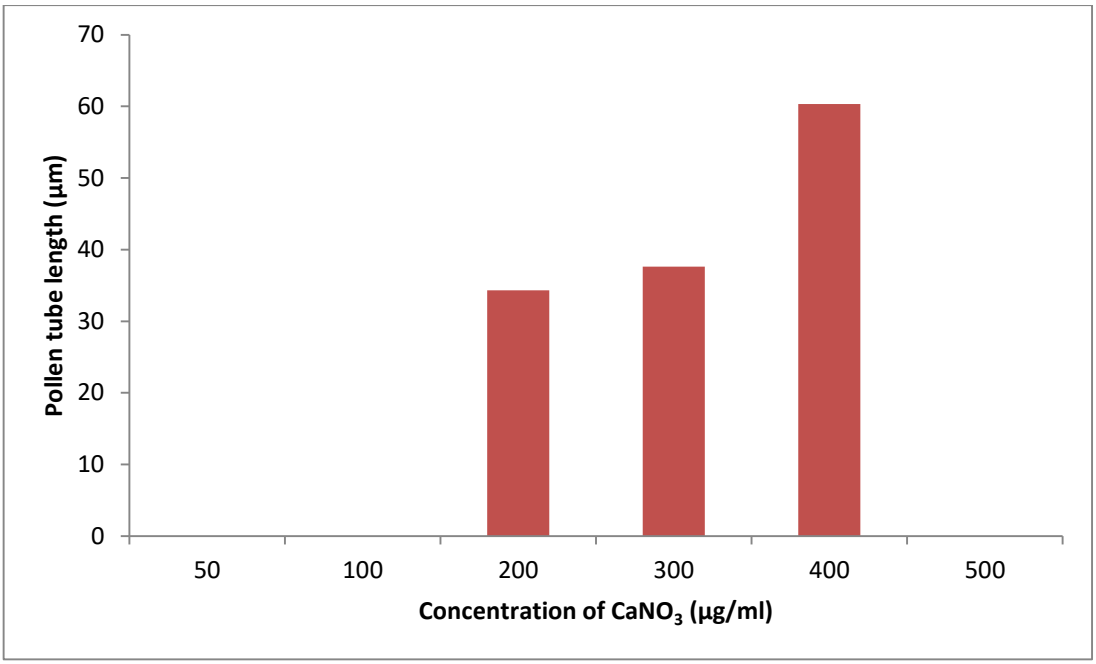
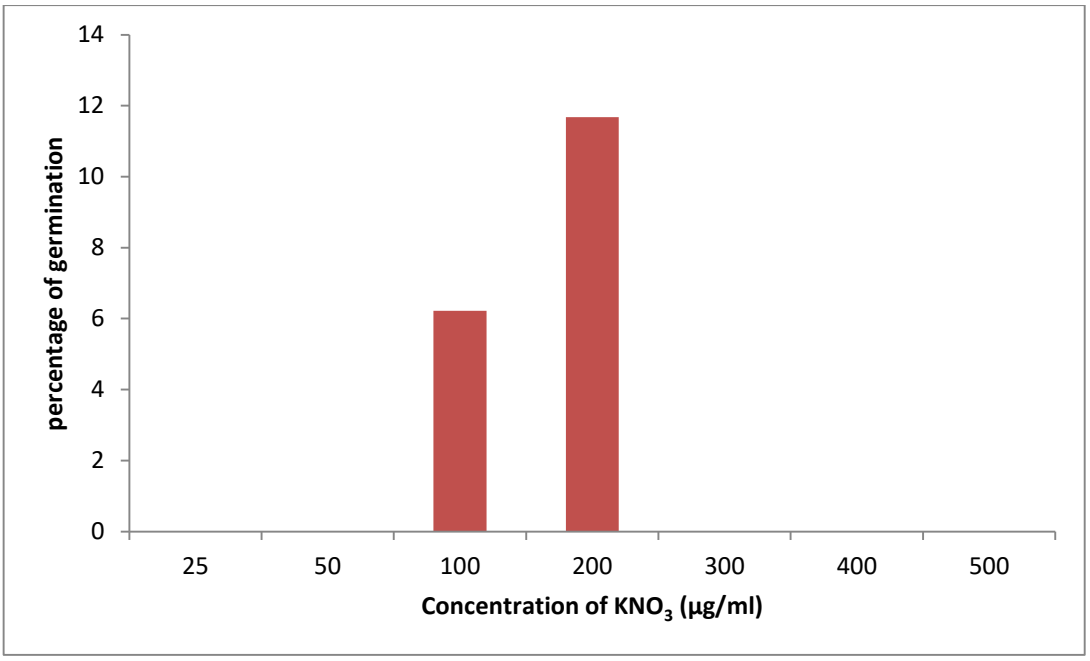


Table 58. *Rhopalephora scaberrima*: In-vitro pollen germination - effect of potassium nitrate

Sl. No.	Concentration of KNO_3 (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	06.22±0.2	38.16±6.2
4	200	11.68±0.68	62.02±3.8
5	300	-	-
6	400	-	-
7	500	-	-

Graph 67. *Rhopalephora scaberrima*: Effect of potassium nitrate - pollen germination



Graph 68. *Rhopalephora scaberrima*: Effect of potassium nitrate - pollen tube length

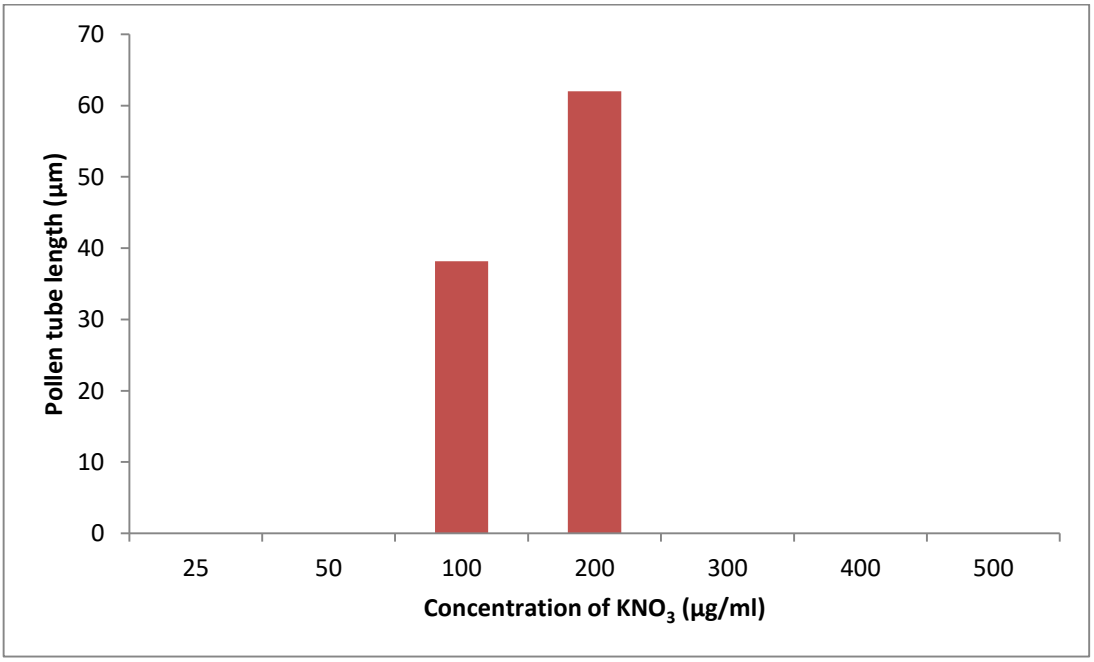
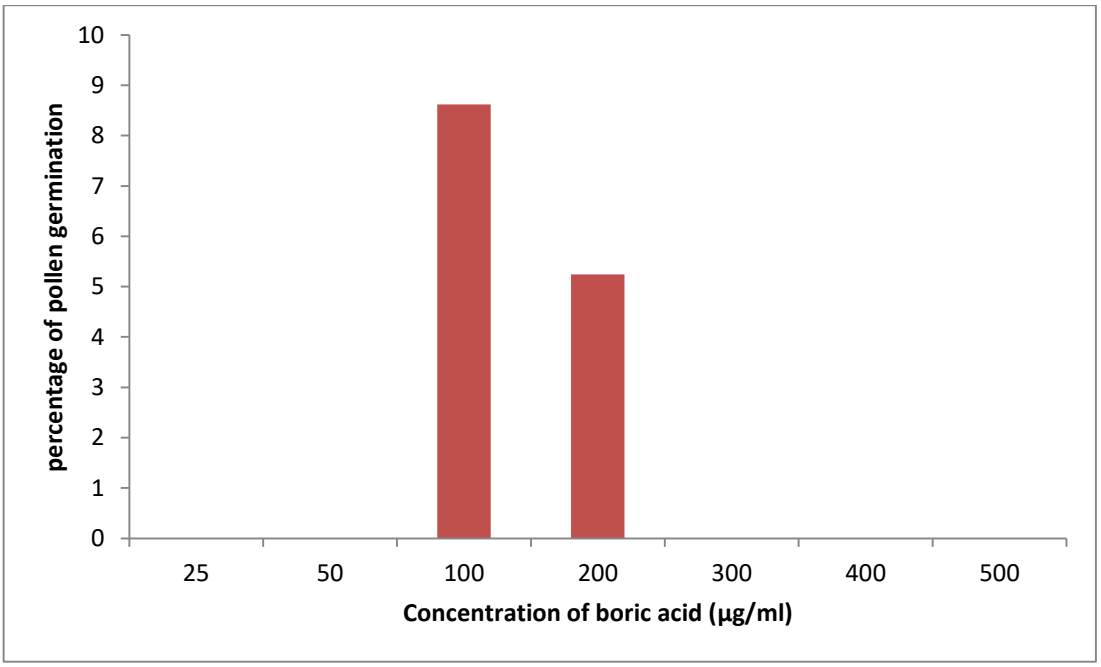


Table 59. *Rhopalephora scaberrima*: In-vitro pollen germination - effect of boric acid

Sl. No.	Concentration of boric acid (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	8.62±0.81	32.07±2.2
4	200	5.24±0.54	28.22±4.2
5	300	-	-
6	400	-	-
7	500	-	-

Graph 69. *Rhopalephora scaberrima*: Effect of boric acid - pollen germination



Graph 70. *Rhopalephora scaberrima*: Effect of boric acid - pollen tube length

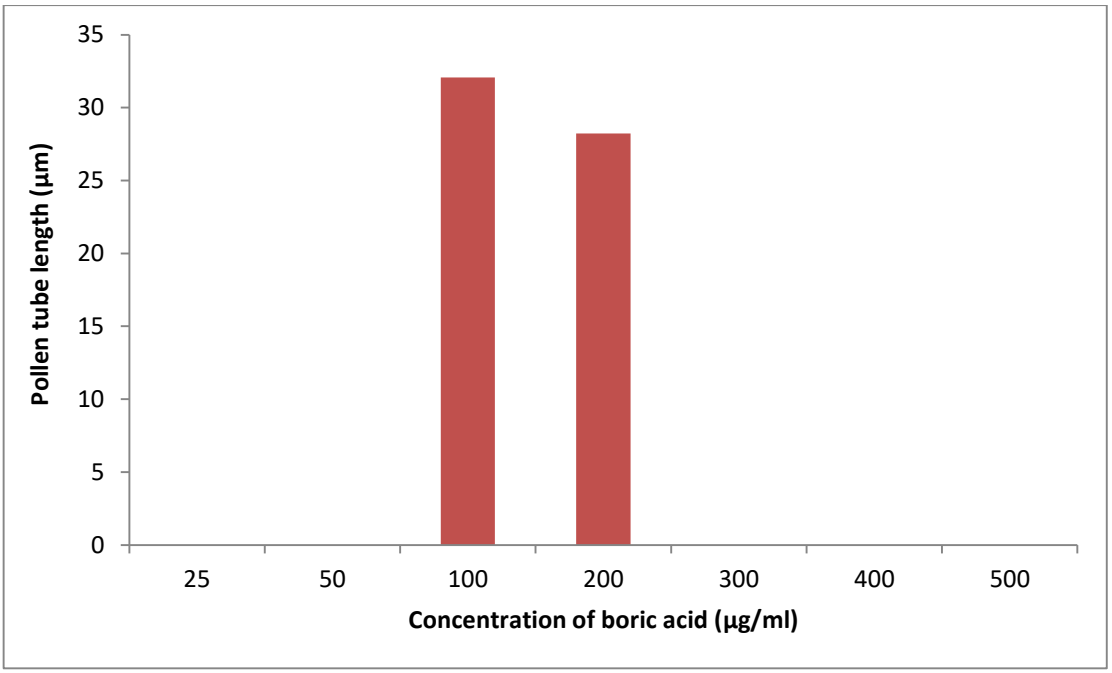
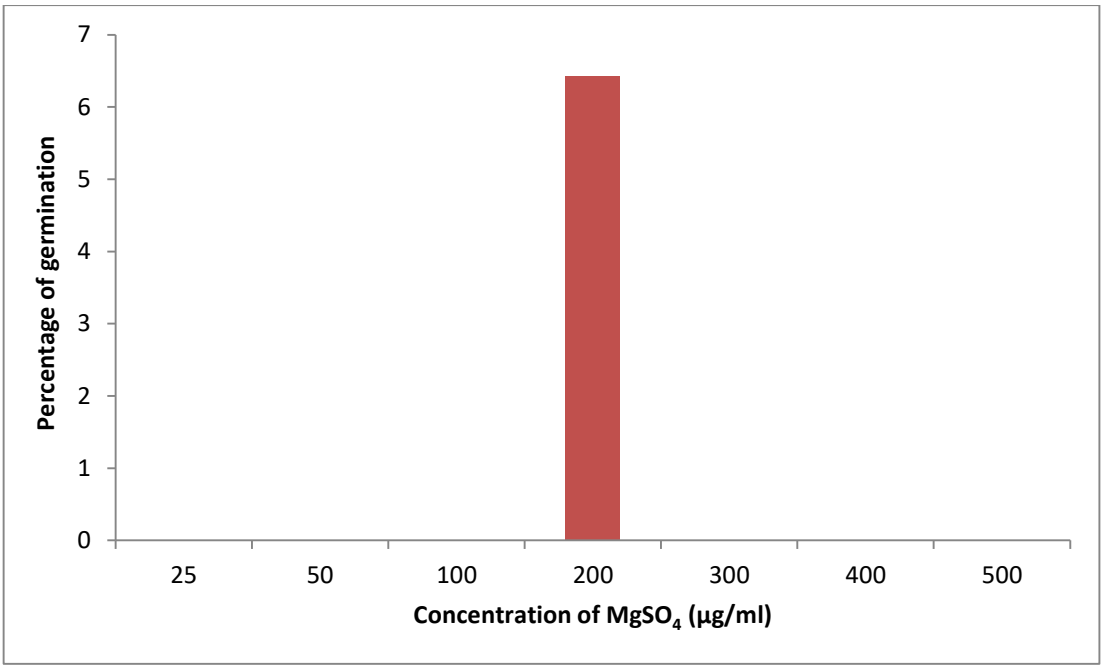


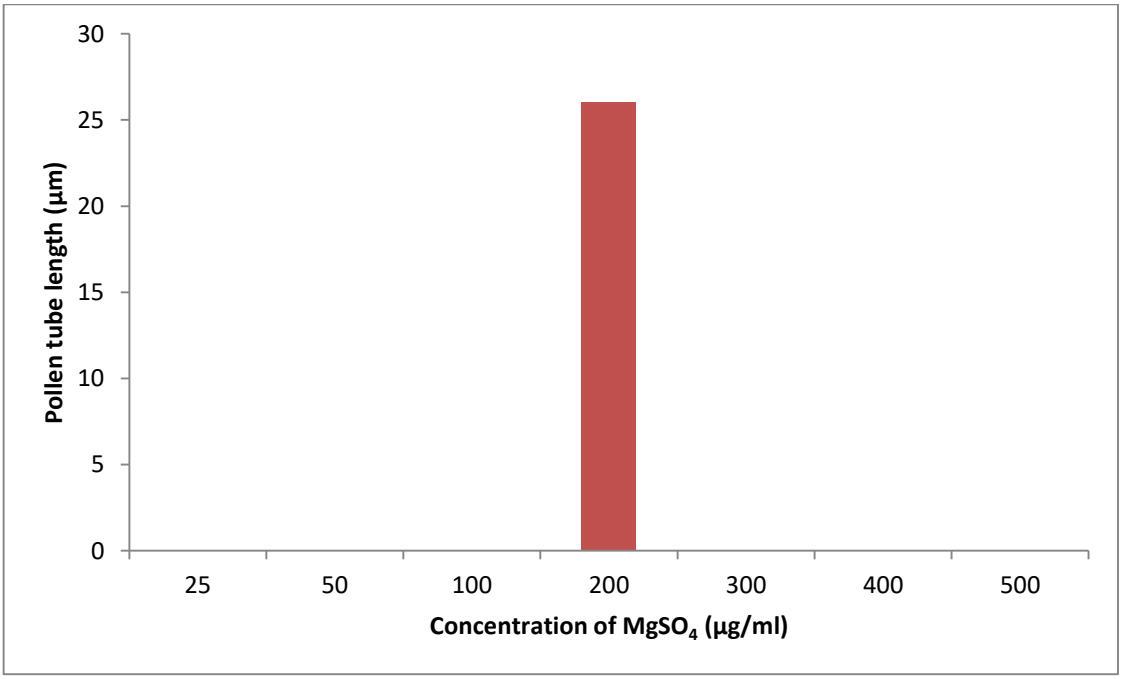
Table 60. *Rhopalephora scaberrima*: In-vitro pollen germination - effect of magnesium sulphate

Sl. No.	Concentration of $MgSO_4$ (µg/ml)	Pollen germination (%) ± S.E.	Pollen tube length (µm) ± S.E.
1	25	-	-
2	50	-	-
3	100	-	-
4	200	6.42±0.13	26.04±6.32
5	300	-	-
6	400	-	-
7	500	-	-

Graph 71. *Rhopalephora scaberrima*: Effect of magnesium sulphate - pollen germination



Graph 72. *Rhopalephora scaberrima*: Effect of magnesium sulphate - pollen tube length



5.5.3 Stigma biology

5.5.3.1 Stigma morphology

The stigma is of dry type and the stigmatic surface measures about 211.27±0.21 μm and the entire surface is covered by papillae of about 117.91±1.8 μm. The style is composed of mostly solid tissue with an axial triangular hollow.

5.5.3.2 Stigma receptivity

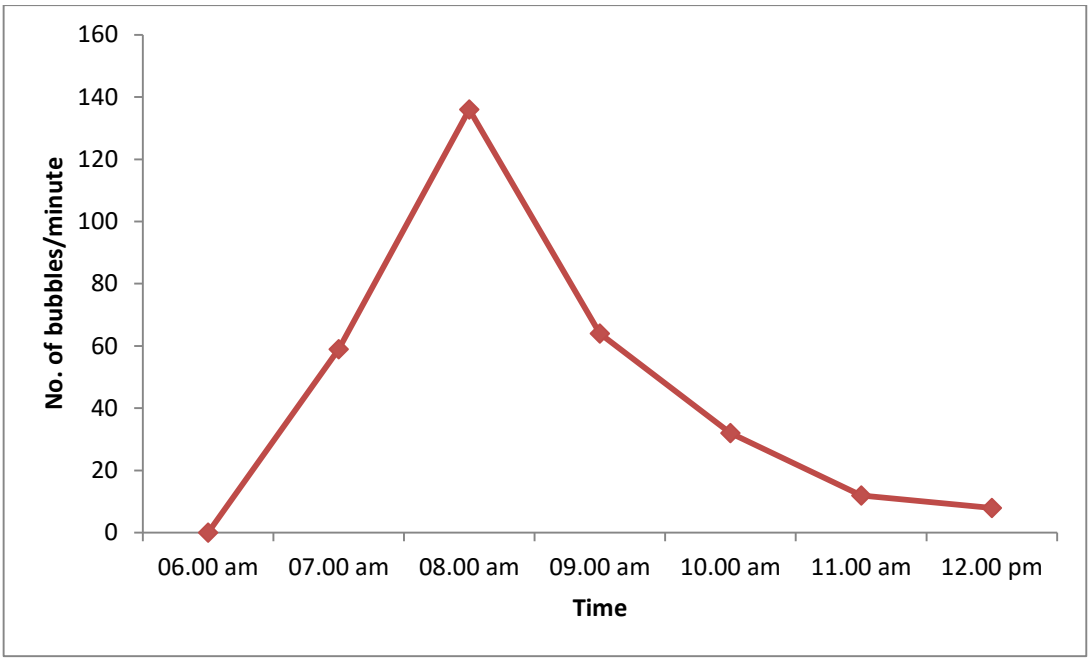
5.5.3.2.1 Stigma receptivity- hydrogen peroxide test

Stigmatic surface showed maximum receptivity around 07.00–09.00 am in hydrogen peroxide test (Fig. 65 C & D; table 61; graph 73).

Table 61. *Rhopalephora scaberrima*: Stigma receptivity - hydrogen peroxide test

Sl. No.	Time of observation	No. of bubbles/minute ± S.E.
1	06.00 am	-
2	07.00 am	59±2
3	08.00 am	136±6
4	09.00 am	64±4
5	10.00 am	32±2
6	11.00 am	12±1
7	12.00 pm	8±2

Graph 73. *Rhopalephora scaberrima*: Stigma receptivity - hydrogen peroxide test



5.5.3.2.2 Cytochemical localization of stigma-surface esterases

Cytochemical localization of stigma-surface esterases using α-naphthyl acetate demonstrated that the stigmatic surface was most receptive during a period between 07.00–09.00 am (Fig. 65 A & B).

5.5.4 Pollination

5.5.4.1 Mode of pollination

Mode of pollination is entomophilous and autogamous.

5.5.4.2 Role of wind in pollination

Examination of the microscopic slides under a microscope revealed an absence of pollen grains thus ruling out wind pollination for the species.

5.5.4.3 Floral visitors and their behaviour

Insect visits started 15–30 minutes after anthesis. *Amegilla zonata* (Apidae), *Tetragonula iridipennis* (Apidae), *Braunsapis* sp. (Apidae), two unidentified species of the family syrphidae (syrphid flies) and *Pelopidas mathias (*Hesperidae) were observed (Fig. 66–69) visiting the flowers of *R. scaberrima*.

Amegilla zonata was observed only rarely and their visits were quick and they moved from flower to flower in seconds. Repeated visits were very rare *T. iridipennis*, *Braunsapis* sp., *Halictus* sp. and the syrphid flies showed similar patterns while foraging. The insects would hand on to the stamens, and work on them. Due to the differences in the filament length between the medial stamen, staminodes and the lateral stamen, the lateral stamens hang lower than the others. It was observed that the after landing on the stamens, these insects invariably reached for the medial stamen and the staminodes first. This places their body in such an angle that their abdomen comes in close contact with the lateral stamen on their flanks and the stigma on the medial part. This positioning seems to enable pollen transfer mostly from the lateral stamens. *T. iridipennis* and *Braunsapis* sp. were sometimes observed to go towards the ovary covered in hairs and then turn around and work on the stamens from an upside down angle. *Pelopidas mathias* were observed occasionally on the flowers, mostly sitting on the petals, without foraging or moving around. More details on the floral visitors are given in the table 62.

Table 62. *Rhopalephora scaberrima*: Floral visitors and their behaviours

Sl. No.	Name of the taxa with family	Foraging	Foraging hours	Time spent on each visit	Stigma touch	Frequency of visit	Found locality		
							Field 1	Field 2	CU campus
1	Unidentified sp. 5 Syrphidae	Pollinator	07.30 am-11.30 am	5-40 seconds	+++	High	✓	✓	✓
2	*Tetragonula iridipennis* Apidae	Pollinator	08.00 am-11.30 am	1-5 seconds	+++	High	✓	✓	✓
3	*Braunsapis* sp. Apidae	Pollinator	08.00 am-11.30 am	5-25 seconds	+++	High	✓	✓	-
4	*Halictus* sp.9 Halictidae	Pollinator	07.00 am-11.00 am	3-10 seconds	++	Intermediare	✓	-	-
5	*Amegilla* sp. Apidae	Pollinator	10.30 pm-11.30 am	4-10 seconds	++	Low	✓	✓	-
6	Unidentified sp. 10 Syrphidae	Pollinator	10.00 pm-11.30 am	3-10 secconds	++	Low	✓	-	-
7	*Pelopidas mathias* Hesperidae	Visitor	Random	2-5 seconds	-	Low	-	✓	-

Stigma touch: +++ very good; ++ good; + poor

Frequency of visits: High (5–30 visits/day); Intermediate (1–5 visits/day); Low (<1 visit/day).

5.5.4.4 Pollination efficiency

Stigmas were observed under a microscope after the flowers closed. Stigmas of 63.33% of flowers showed the presence of pollen.

To study the pollination efficiency by individual visitors, stigmas were observed right after the first visit. It was found that unidentified sp. 5 (Fig. 69) and *Tetragonula iridipennis* (Fig.67) were the most efficient pollinator of *R. scaberrima.*

5.5.5 Breeding system

The fruit set and seed set percentages from each of the pollination treatments are shown in the tables 63 & 64 and graphs 74 & 75.

5.5.5.1 Apomixis

No fruit set was observed in flowers bagged after emasculation, prior to anthesis.

5.5.5.2 Autogamy

Fruit set in flowers bagged before anthesis was 42.86% and 40% at population 1 and population 2 respectively.

5.5.5.3 Manual self-pollination (MSP)

Pollinating flowers by pollen from the same flowers showed 85.71% of fruit set in population 1 and 86.67% in population 2.

5.5.5.4 Manual cross-pollination (MCP)

Flowers pollinated by pollen from other plants showed 94.29% of fruit set in population 1 and 90% of fruit set in population 2.

5.5.5.5 Open pollination (OP)

Flowers left to be pollinated by natural pollinators under natural conditions showed 51.42% of fruit set in population 1 and 56.67% in population 2.

Table 63. *Rhopalephora scaberrima*: Breeding system, Population 1

Sl. No.	Treatments	POP 1 Adivaram					
		No. of flowers treated	No. of fruits developed	Percen-tage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seed set
1	Apomixis	35	0	0	0	3	0
2	Autogamy	35	15	42.86	28	3	26.67
3	M S P	35	30	85.7 1	61	3	58.09
4	MCP	35	33	94.29	66	3	62.85
5	OP	35	18	51.42	36	3	34.29

Graph 74. *Rhopalephora scaberrima*: Breeding system - fruit set and seed set at population 1

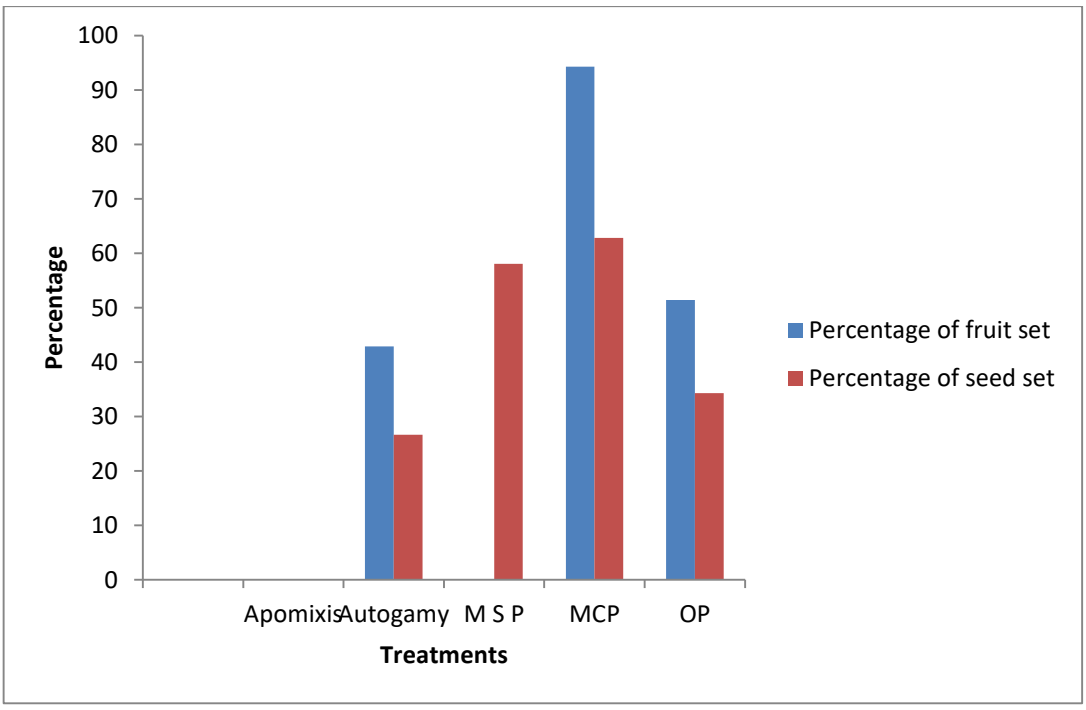
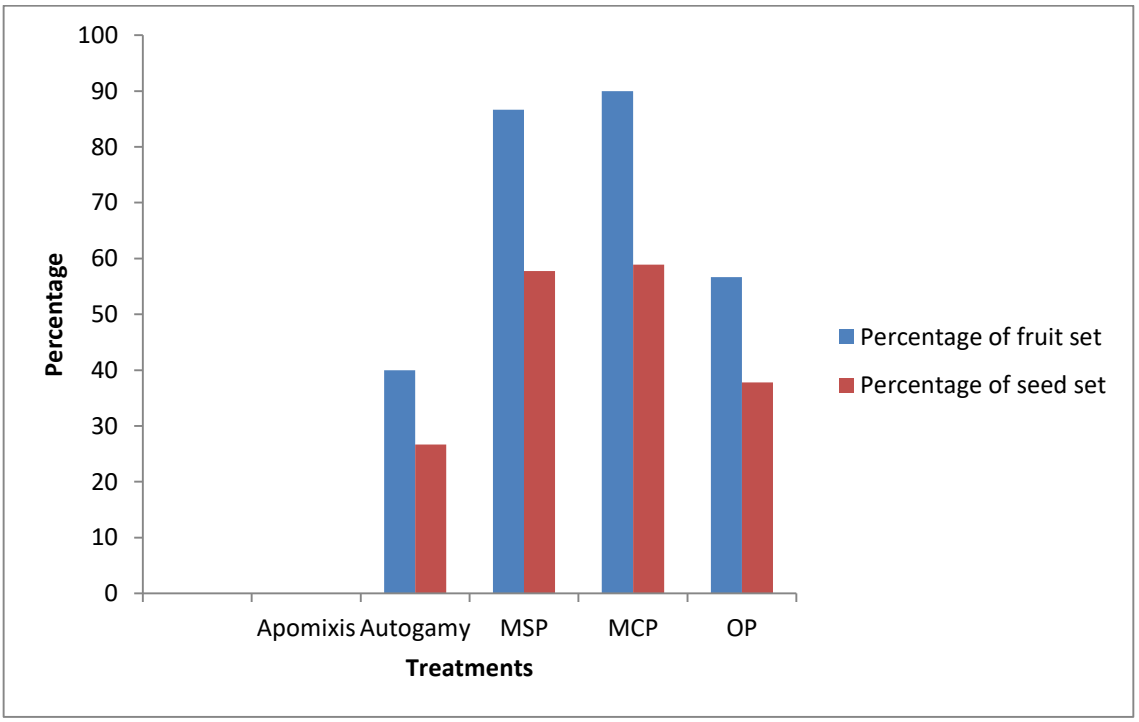


Table 64. *Rhopalephora scaberrima*: Breeding system, Population 2

Sl. No.	Treatments	POP 2 Vellanipacha					
		No. of flowers treated	No. of fruits developed	Percentage of fruit set	No. of seeds	No. of ovules per flower	Percentage of seed set
1	Apomixis	30	0	0	0	3	0
2	Autogamy	30	12	40.00	24	3	26.67
3	MSP	30	26	86.67	52	3	57.78
4	MCP	30	27	90.00	53	3	58.89
5	OP	30	17	56.67	34	3	37.78

Graph 75. *Rhopalephora scaberrima*: Breeding system - fruit set and seed set at population 2



5.5.6 Fruit and seed biology

After fertilization the petals fall off from the flower and the sepals and style persists. A long persistent style was found crowning the capsule. The fruits matured 3–4 days after fertilization and the capsules dehisced 6–7 days after

fertilization. The presence of numerous, uncinate (hooked) hairs make the capsule sticky and facilitate the dispersal of the seeds by sticking on the body surface of animals that come in contact with these plants. Capsules are subglobose, coriaceous, trilocular with a flattened ventral locule, and inflated dorsal locules. Capsules dehisce along the ventral locule (Fig. 63 E-H).

Seeds are 2, sometimes 1 and rarely 3, grey-blue, trapezoidal in outline, ventral surface planar and the dorsal surface convex. Embryotega is lateral on the ventral surface and hilum is linear on the dorsal surface. Seed surface ornamented with irregular ridges radiating from the embryotega and polygonate cells with depressed edges and smooth projecting faces.

5.5.7 Flower-fruit ratio and ovule-seed ratio

Under natural conditions, the flower to fruit set ratio was 13:7 and the ovule to seed set ratio was 39:14.

5.5.8 Seed germination

Mature seeds kept in a petri-dish over cotton soaked in water showed 9% germination whereas seeds sowed in pots under nursery condition showed 32% germination.

Rhopalephora scaberrima shows hypogeal and remote tubular germination pattern where a non haustorial part of the cotyledonary hyperphyll (apocole) grows out of the seed and create some distance between the seed and the cotyledonary sheath. In this species, the apocole is attached with the cotyledonary sheath at the apical region (Fig. 70).

Table 65. *Rhopalephora scaberrima*: Summary of floral characters

Sl. No.	Floral characters	Observations		
1	Flowering period	July to January		
2	Flower type	Zygomorphic, bisexual		
3	Flower colour	Pale lilac		
4	Odour	Absent		
5	Nectar	Absent		
6	Anthesis time	6.30-07.00 am		
7	Anther dehiscence time	6.35-07.10 am		
8	Anther dehiscence	Longitudinal		
9	No. of anthers/flower	3		
10	No. of staminodes/flower	2 (rarely 3)		
11	Mean no. of pollen grains/ anther	1678±228 (L) 2791±127 (M)		
12	Mean no. of pollen grains/ flower	6148		
13	Mean no. of ovules/flower	3		
14	Pollen- ovule ratio	6148:3		
15	Pollen structure	Spinulose and monolete		
16	Pollen size		P	E
		L	70.99±0.67µm	33.62±0.29µm
		M	68.53±0.61µm	29.30±0.30µm
17	Pollen shape	Perprolate		
18	Stigma type	Dry, papillate		
19	Pollen viability (max%)	87.51±4.53 (L)		
20	Fruit type	Capsule		
21	Flower-fruit ratio	13:7		
22	Ovule-seed ratio	39:14		
23	Flower closing time	11.30 am–12.00 pm		

6. DISCUSSION

The five species selected for the study, *Commelina diffusa, Dictyospermum montanum, Floscopa scandens*, *Murdania nudiflora* and *Rhopalephora scaberrima* belongs to the family Commelinaceae (sub-family Commelinoideae; tribe Commelineae). These species are mostly considered as troublesome weeds (Moody, 1989; Waterhouse, 1993) but are also used as vegetables, fodder or even used as medicine in some parts of the world (Burkill, 1985). Irrespective of their utility, they are an essential part of our ecosystem.

Organisms and their environment are interdependent and organisms respond to the changes in their environment by modifying their behaviour. Phenology is the study of the timing of life cycle phases or recurring biological events occurring in a plant. Population phenological studies help us to understand the long term adaptation strategies of species to the current and changing environmental conditions whereas studies on floral pheno-events help us to understand the relationships between plants and animal visitors (Shivanna & Tandon, 2014).

6.1 Phenology

Of the five selected species, two were annuals (*C. diffusa* and *M. nudiflora*) and three perennials (*D. montanum*, *F. scandens* and *R. scaberrima*). All five are found commonly near rivers or streams (even sewers in the case of *C. diffusa*). Growth and flowering periods of all but one (*F. scandens*), initiates with the rainy season and ends shortly after it. Variations in pheno-events were observed with changes in climatic variables as suggested by

Sigrist and Sazima (2015). The duration of flowering for *R. scaberrima* was successfully extended under greenhouse condition.

The investigated taxa showed similarities in anthesis timing and patterns. While the flowers of *R. scaberrima* and *C. diffusa* opened in early morning (6.30–07.30 am), *D. montanum* flowers opened at mid-morning (09.30–10.00 am) and the flowers of *M. nudiflora* and *F. scandens* opened only towards the latter part of the morning (10.30–11.30 am). Anther dehiscence takes place either with or a few minutes after anthesis. Pacini (1992) also observed that the anthers may dehisce before, after or at the same time as the flower. Similar to the observations of Huang *et al*. (2004), the dehiscence was generally synchronous in natural populations. Mode of anther dehiscence was of longitudinal type in all selected species. According to Keijzer *et al*. (1987) the two types of anthesis occurring during blooming (of the flower and the anther), are not always simultaneous, and bisexual flowers are often dichogamous. But in the selected species, only *D. montanum* showed slight protogyny, while in all others the androecium and gynoecium were receptive simultaneously. Nepi and Pacini (1993) recorded that the end of anthesis is usually accompanied by the end of receptivity of one or both sexes. This holds for most of the species studied where receptivity of both sexes became close to zero by the end of anthesis except for *C. diffusa*, where both sexes were receptive during closing.

6.2 Pollen biology

Dafni and Firmage (2000) consider pollen viability as an important parameter of pollen quality. Pollen viability testing using TTC solution is one of the most common and popular methods in use (Mulugeta *et al.*, 1994; Shirazi & Muir, 1998; Dafni & Firmage, 2000). The maximum viability periods of the selected taxa ranges from 07.00 am–12.00 pm. It is found that pollen from the different types of anthers was capable of siring seeds (except in

R. scaberrima). A similar observation was also made by Hrycan and Davis (2005) in *C. dianthifolia* and *C. coelestis*, where the pollen from the medial anthers was viable. They suggest that in these species the pollen from medial anthers does not have function in just attraction and reward but also in pollination.

Pollen germination was observed to be affected by the concentrations of calcium nitrate, potassium nitrate, boric acid, magnesium sulphate and sucrose in the germinating medium. Brewbaker and Kwack's medium was found to give the maximum germination and pollen tube growth. Sucrose solution of 10% concentration gave the next best results for all the studied species except for *D. montanum*, which showed better results in 3% sucrose solution. Germination in other nutrient mediums were incidental but the best concentrations of each nutrient was identified.

Pollen morphological characteristics are displayed in the outermost wall layer, the exine. The ornamentations on the exine along with number, position and characters of the aperture have been used to classify the pollen grains and are very useful in systematic studies. Like most members of the family Commelinaceae, pollen grains of the studied species showed similar surface morphology and tectal ornamentations (Poole & Hunt, 1980). *R. scaberrima* stood out from the others regarding the pollen being perprolate in shape while all the others were prolate.

Cruden (1977) correlates the amount of pollen produced by a flower to the probability that, the pollen will reach the stigma. He describes pollen to ovule ratios (P/O) as a conservative indicator of pollination mechanism and breeding systems in a plant. He claims that a lower P/O ratio indicates a more efficient pollen transfer mechanism and concludes that cleistogamous flowers would have the lowest P/O ratios and an obligate xenogamous species to have very high P/O's. The lowest P/O ratio in our study was found in *M. nudiflora*

(266.5) and according to Cruden (*l.c.*), the ratio is indicative of facultative autogamy. This is in congruence with the finding of facultative cleistogamy in this species by Veena and Nampy (2019). In *C. diffusa*, bisexual flowers of the third-order showed the lowest P/O (593) indicative of facultative autogamy while the flowers of the first-order showed a slightly higher P/O (1508) indicative of facultative xenogamy. P/O ratio of *F. scandens* (919) also indicated facultative xenogamy while that of *R. scaberrima* (2049.33) and *D. montanum* (3144) indicated predominant xenogamy. Many other works have also shown that the flowers of self-incompatible and xenogamous taxa produce more pollen grains than those of self-compatible and/ or autogamous taxa (Arroyo, 1973; Baker, 1967; Cruden, 1973; Gibbs *et al*., 1975; Lloyd, 1965; De Vries, 1974).

6.3 Floral biology

Cruden (1977) also observed facultatively xenogamous species to be regularly self-compatible and protogynous or homogamous. He also noted that although some species require a pollinator, most self-pollinate when the flowers close. In such species, though selfing may take place, the adaptations favour xenogamy and selfing occur mostly in the absence of, or in addition to outcrossing. This is notable in the case of *C. diffusa*, where the inflorescence, floral structure, anther dimorphism, andromonoecy, etc., seem to favour outcrossing; the bending of the staminal filaments and the coiling of the style seems to favour selfing. Hrycan and Davis (2005) and Kaul and Koul (2008) made similar observations in *C. dianthifolia* and *C. caroliniana* respectively. Thus autogamous and self-compatible species might have adaptations that favour xenogamy (Cruden, *l.c.*). Simpson *et al*. (1986) suggested that in species where mimicry is used, autogamy must be delayed and facultative to first allow the possibility of cross-pollination enabled by the pollinators. Delayed selfing or autogamy was also observed in *C. benghalensis* and

C. forskaolii (Maheshwari & Maheshwari, 1955), *C. coelistis* (Faegri & Van der Pijl, 1979), C. *communis* (Ushimaru *et al*., 2003a), *C. erecta* (McCollum *et al*., 1984) and in *C. dianthifolia* (Hrycan & Davis, 2005).

Murdannia nudiflora shows facultative cleistogamy as an adaptive response to certain environmental conditions (Keighery, 1982; Veena & Nampy, 2019). Keighery (1982) also reported cleistogamous flowers in *M. nudiflora* as a response to rain but he failed to observe any insect pollinators visiting the species. In contrast, it is found that cleistogamy in this species function as a part of a mixed mating system, thereby providing more chances of survival. Cleistogamy in response to environmental conditions has been observed in *C. benghalensis*, *C. forskaolii* (Maheshwari & Maheshwari, 1955) and *C. dianthifolia* (Hrycan & Davis, 2005).

Of the five selected taxa, *C. diffusa, D. montanum*, *R. scaberrima* and *F. scandens* show heterantherous condition or anther dimorphism. According to Faden (2000), heteranthery is a means of floral deception and the different types of stamens may differ in filament length, curvature, anther size, shape, colour, pollen amount, size, structure etc. It is found that other than these characters, they also differ in the viability of the pollen produced. In *R. scaberrima*, only the lateral stamen produces viable pollen grains, while in all the other species the pollen produced by the lateral stamens were significantly more viable and showed higher percentages of germination than those produced by the medial stamen. Stamens producing only sterile pollen were also reported in *Palisota* spp. and *Tripogandra* spp. (Handlos, 1970, 1975). Decraene and Smets (2001) observed that pollen produced by the feeding stamen in heterantherous flowers is usually non-viable whereas Hrycan and Davis (2005) found they produced viable pollen in *C. coelestis* and *C. dianthifolia*.

The various floral organs, especially those positioned in the upper half of *Commelina* flowers are known to serve the function of attraction and deception of pollinators. The upper half of the flower typically comprises two showy petals and three showy staminodes (Vogel, 1978; Dafni, 1984; Faden, 1992, 2000; Endress, 1994) along with the medial stamen in the case of heterantherous species. This is seen in *R. scaberrima* and *C. diffusa* with asymmetric petals and staminodes and also for *F. scandens* in which the anthers concerned with attraction is oriented at the upper half of the flowers. The medial stamen, typically oriented near the staminodes (if present) producing less viable pollen is primarily concerned with attracting and rewarding the pollinators (the feeding stamen, Vogel, 1993) whereas the lateral stamens are concerned with pollination (pollination stamen, Vogel, 1993). Heteranthery indicates the division of labour between stamens modified for pollinator attraction and those producing pollen for pollination (Vogel, 1978; Faden, 1992).

Ushimaru *et al*. (2007) and Veena and Nampy (2020) experimentally verified this theorized pollination syndrome for some members of the family Commelinaceae. Following the experimental removal of various floral organs in different flowers, under different conditions, they both evaluated the pollinator responses to these changes and their effect in fruit sets. Ushimaru *et al*. (*l.c*.) provided evidence that the two large blue upper petals of *C. communis* do, in fact, attract potential pollinators and also demonstrated that the bright non-rewarding staminodes, as well as the medial stamen, serve not only to attract but also to guide pollinators to land and promote efficient pollen transfer. Veena and Nampy (2020) demonstrated the functional differences in dimorphic stamens of *C. diffusa, D. montanum* and *R. scaberrima* and also explained their role in efficient pollen transfer, utilizing the 'safe sites' on the body of pollinators. Kaul and Koul (2012) observed that in *C. benghalensis* and *C. caroliniana*, the pollinators first

looked for and probed the medial anther and then went to the lateral anthers. During this process they get dusted with pollen grains, especially from the lateral stamen, and when the insect visit another flower, their abdomen, carrying that pollen, brushes against the stigma.

Andromonoecy, as found in *C. diffusa*, is known to be very common in the family Commelinaceae (Maheshwari & Maheshwari, 1955; McCollum *et al*., 1984; Faden, 2000). It is observed that the fruit set of the first and second-order flowers in *C. diffusa* appeared to cause the retardation of gynoecium development in the third flower of the same spathe. Maheshwari and Maheshwari, (1955) and McCollum *et al*. (1984) also found that pollination in the initial flowers caused abortion of the gynoecium in subsequent ones in *C. forskaolii* and *C. erecta* respectively. The number of fruits in an andromonoecious species developing within an inflorescence has an effect on the gender of subsequent flowers within that inflorescence (Diggle, 1991). Though the mechanism causing female sterility is unknown, it is evident that the sexuality of the lower order flowers are influenced by fruit set in the higher-order flowers.

The flowers in Commelinaceae have a very short period of longevity and limited resources to offer to the pollinators. Nectarless flowers with only pollen to provide as a reward to pollinators often uses deception to attract the pollinators (Vogel, 1978). Persistence of the faded flowers even after failing to set fruit as observed in *F. scandens* creates a visual impact of a dense inflorescence (Faden, 1992). The dense sepaline hairs in *F. scandens* make the flowers extremely showy and are possibly involved in pollination (Faden, *l.c*.). Filament hairs found commonly in this family was present only in one of the selected species, *M. nudiflora*. They are described to work in a multitude of ways such as, attracting insects towards or away from the main pollen source, affecting the movement of insects within a flower, how they collect

pollen etc., (Faden, *l.c*.). A higher number of fruit set percentages and insect visitors in emasculated flowers retaining their bearded filaments as compared to completely emasculated flowers in *M. nudiflora* confirms the deceptive potential of the filament hairs (Veena & Nampy, 2019).

In *R. scaberrima* all floral parts, except the ovary is glabrous. These hooked glandular trichomes did not seem to have any secretory function or odour. A similar situation was found in *C. dianthifolia* (Hrycan & Davies, 2005). Faden (1992) proposed that these hairs might be protective in function but it seems that in *R. scaberrima*, it also functions to facilitate seed dispersal. The hooked hairs retained on the fruit helps attach the capsules on to a passer-by (person or animal).

6.4 Stigma biology

In all the five species studied, the stigma surface is covered by papillae and there is no visible opening to the stylar canal. In all species except *R. scaberrima*, the style was found to be solid while in *R. scaberrima* it was fistular. Also, no apparent secretion was found in the stigmatic surface in any of the species under consideration. Owens and Kimmins (1981) state that several species of *Aneilema* and *Commelina* possess dry stigmatic surfaces though it is not true for all species in these genera. According to Owens and Kimmins (*l.c*.) the stigma size ranged between 0.1–1.3 mm in 17 genera and 68 species of this family surveyed, except in *Callisia*. In this study, it is found that the stigma ranged between 0.073–0.2 mm.

Mirror-image flowers were first reported by Todd (1882), who observed flowers with left and right deflected styles. The term "enantiostyly" was later on proposed by Knuth (1906), who defined it as "the occurrence of right-styled and left-styled flowers". Jesson *et al*. (2003a) reported many species with a single stamen deflected to the opposite side of the flower to the style

and the condition was termed "reciprocal enantiostyly". There are two different patterns of organization of left and right-handed flowers on individual plants (Jesson *et al*., 2003a), the "monomorphic enantiostyly" and "dimorphic enantiostyly." Monomorphic (presence of left- and right-styled flowers on the same plant) reciprocal enantiostyly is found in *D. montanum.*

Enantiostyly is widely distributed in both dicots and monocots and is documented in a minimum of 10 angiosperm families, belonging to eight orders. All enantiostylous taxa are animal pollinated, mostly by insects, particularly pollen-collecting bees (Jesson *et al*., 2003b). Sometimes in enantiostylous species, the deflection of a stamen is not accompanied with stylar deflection. This condition is very rare and has so far only been reported in, *Murdannia* of the family Commelinaceae (Evans *et al*., 2000).

Enantiostyly was suggested to be a part of a floral syndrome by Dulberger (1981). He proposed that the floral syndrome was associated with traits such as, nectarless flowers with heteranthery or anther dimorphism, the orientation of anthers leading to deposition of pollen from the medial "feeding" anthers on the ventral parts of the insect and of the "pollinating" anthers on dorsal parts, curved styles and stigmas touching the zone on which pollen has been deposited from the "pollinating" anthers, minute stigmas etc. All these traits were found in *D. montanum*. Graham and Barrett (1995) suggested the presence of a pollination syndrome in which the consistent positioning of insect pollinators is important for effective cross-pollen transfer as the reason for the frequent association of these traits and enantiostyly. However, it was reported that in reciprocal monomorphic enantiostyly, a pollinator can visit alternate flower forms on the same plant, causing geitonogamous self-pollination (Helme & Linder, 1992; Fenster, 1995; Graham & Barrett, 1995). But when compared to non-enantiostylous plants, monomorphic enantiostyly

may reduce the levels of geitonogamy (Dulberger & Ornduff, 1980; Barrett *et al*., 2000).

6.5 Pollination biology

Wind pollination in the studied species was ruled out after the results from hanging slide experiments. Kaul and Koul (2012) also observed that *C. caroliniana* and *C. benghalensis* does not adopt wind pollination. These plants, with the disadvantage of a lack of any reward other than pollen for pollinators attract an array of insects by means of their colourful petals, hairy filaments or sepals, staminodes, broad connectives, dimorphic anthers, etc., (Faden, 2000). The flowers were visited by a number of hymenopterans and depterans and an occasional blattodean. Among the five taxa, many of the visitors including, *Amegilla zonata*, *Tetragonula iridipennis*, *Halictus* sp., *Ceratina* sp. etc., were common. Several species from the family Syrphidae, commonly called as syrphid flies, were also found visiting the five taxa. Though the flower structure allows an unrestricted access to a wide range of visitors, the pollinator species of these taxa are dominated by syrphid flies, halictid bees and apid bees. Williams and Walker (2003) found that in *Pollia crispata*, syrphid flies, halictid bees and *Trigona carbonaria* formed the major pollinators. *Commelina cyanea* is recorded to be pollinated by the apids *Amegilla pulchra*, *Nomia aurantifer* and the syrphid fly, *Syritta* sp. (Williams, 1993).

Despite the differences in their size, floral visitors showed general behavioural patterns. Kaul and Koul (2012) had found that the insect visitors first alighted on the male flowers and then moved on to the bisexual ones in *C. caroliniana* and *C. benghalensis*. In the andromonoecious species of *C. diffusa*, a similar pattern was found during the initial visits but it was also noted that the visiting patterns became more random on the repeated visits.

After landing on the flowers, the insects invariably went for the medial stamen first and probed those with their legs and mouth and then moved to the lateral stamen. When this insect visited another flower the ventral side of their abdomen and legs (and sometimes even the dorsal surface) laden with pollen, especially from the lateral stamens, came in contact with the stigma (Kaul & Koul, 2012). The positioning of the stigma, facing the medial stamen in most of the species studied seems to enable this process and thereby bring the purpose of the division of labour within the stamens to fruit. Veena and Nampy (2020) found that the percentage of fruit set by pollen from the medial stamen were lower than those by pollen from the lateral stamen. A decrease in the percentage of fruit sets when flowers were allowed to be pollinated naturally after removing their medial stamen were also observed in these species.

6.6 Breeding system and seed germination

Like most members of this family (Maheshwari & Maheshwari, 1955), the species selected under study was self-compatible and as floral mimicry is employed, autogamy was found to be either facultative or delayed (Simpson *et al*., 1986), in order to allow for cross-pollination by the entomophilous pollinators (Hrycan & Davis, 2005). High percentages of autogamous fruit set were observed in *C. diffusa* (72.38%), *M. nudiflora* (47.62%) and *R. scaberrima* (41.43%) while *D. montanum* (2.5%) and *F. scandens* (14%) showed relatively low percentages. *D. montanum* seems to favour cross-pollination with a high percentage of fruit set in manually cross-pollinated flowers (76.75%) and slightly lower percentage in manually self-pollinated flowers (50.25%). Similarly *F. scandens* also showed a higher percentage of fruit set when manually cross-pollinated (84%) and a comparatively lesser percentage when manually selfed (70%). But in the other three species manuall crossing and selfing showed no obvious differences in fruit set

percentages. Hrycan and Davis (2005) also reported that there was no significant differences in fruit set of manually selfed and crossed flowers of *C. coelestis* and *C. dianthifolia*.

Seed germination occurred under natural and nursery conditions for all the selected taxa but under invitro conditions, both *D. montanum* and *F. scandens* were unable to germinate. *C. diffusa*, *M. nudiflora* and *R. scaberrima* showed hypogeal germination. In *C. diffusa* and *R. scaberrima* remote tubular germination pattern was observed. In such germination an elongated, nonhaustorial part of the apocole or cotyledonary hyperphyll presents a distance between the germinating seed and the cotyledonary sheath. Bose and Paria (2019) observed similar patterns of germination in *C. benghalensis*, *C. caroliniana* and *C. paludosa*. He also noted differences in the cotyledonary sheath, apocole, first leaf, etc.

As Williams and Walker (2003) observed in *Pollia crispata*, in the species selected under study also, the plant-pollinator interactions are typical of the interactions documented from the understory flora of rainforests where the plant-pollinator interactions are more specialized and the pollinator diversity for individual plants are limited (Hamilton, 1897; Williams & Adam, 1994, Williams *et al*., 2001). Rainforest understories are also known to be vulnerable to destructions caused by clearing for paths, roads, recreational activities or by the invasion of exotic weeds (Williams & Gerrand, 1998; Williams & Adam, 1999). However, the species under consideration is not completely dependent on pollinators alone, they are self-compatible and as discussed earlier they have developed various mechanisms for self-pollination.

Observations of insect landings and behaviour on flowers also suggest that floral traits including the variation in perianth structure, relative positions of sexual organs, the location of the reward, hairs, etc., influence the position of

the pollinators and control the amount and efficiency of pollen removal, transfer, and deposition (Robertson & Lloyd, 1991; Harder & Barrett, 1993). Except for *D. montanum* no obvious differance in seed set was found between selfed and crossed flowers (Hrycan & Davis, 2005). This along with the P/O ratios, floral structure and occurrence of mirror-image flowers, again reinforces the idea of predominant xenogamy in *D. montanum*. The evolutionary shift from xenogamy (outcrossing) to autogamy (selfing) has been mediated through decreased flower size and alterations in floral morphology (Ornduff, 1969). Self-compatibility, contrivances for delayed or early selfing and adaptations to attract pollinators together contributes to the high reproductive efficiency of these species.

7. SUMMARY AND CONCLUSION

Sexual reproduction in plants acts as ploy to achieve maximum reproductive success and genetic diversity. Through millions of years of evolutionary selection and rejection processes, each plant group has evolved different strategies to achieve optimal reproductive success. Although the enthusiasm for the field of reproductive biology has increased enormously over the last few decades, pioneering studies in this field have been very limited throughout the world and especially in India.

Pollination biology of five selected species of the family Commelinaceae (tribe Commelineae) namely *Commelina diffusa* Burm.f., *Dictyospermum montanum* Wight, *Floscopa scandens* Lour., *Murdannia nudiflora* (L.) Brenan and *Rhopalephora scaberrima* (Blume) Faden has been documented in this study. Studies on the reproductive biology of this family have been extremely limited. These species are found extensively in Kerala and adequately represent a wide range of the characteristics of the family. With highly evolved mechanisms to ensure survival and adaptations to enhance reproductive potential with minimal expenditure of resources, they form one of the ideal systems to study reproductive biology. Detailed knowledge of the reproductive mechanisms of the members of such a diverse family not only illustrates the possible measures of control and conservation for these species but also provides an accurate idea of the various adaptive mechanisms in reproduction that plants in general use to survive.

In order to assess the reproductive mechanisms of the selected species, all the major aspects of pollination biology, including the flowering phenology, floral biology, pollen morphology, pollen viability, stigma morphology, stigma receptivity, breeding system, the mechanisms of pollination and

reproductive efficiency were evaluated. The study was carried out during 2015-2019 both in laboratory and in the field and this is the first detailed record of the pollination biology of the selected taxa. Various experiments were carried out and the most suitable protocols were standardized for evaluating the aforementioned parameters. The period of flowering in a population and the duration of flower opening and various other pheno-events play an important role in facilitating successful reproduction and optimizing fitness. All the selected species of the 'day flower family' had very short duration of flower opening with less than a day of flower longevity. The more or less simultaneous periods of viability of both male and female reproductive structures ensure a chance of successful pollination in these taxa. Floral architecture and the design of various floral whorls play an important role in determining the successful interactions between the male reproductive structures, the pollinators and the female reproductive structures. Heteranthery was found in *Commelina diffusa, Dictyospermum montanum* and *Rhopalephora scaberrima*. Presence of dimorphic or heterantherous stamens in these taxa represents mechanisms to ensure optimal pollen transfer to stigmas in flowers that are limited with only pollen as a reward. Enantiostyly, as found in *Dictyospermum montanum*, is an adaptive modification to enhance the pollen export and to increase cross pollen transfer. Deceptive floral hairs, coloured anthers, conspicuous inflorescences, etc., also played important roles in floral attraction in the selected species.

Evaluation of the pollen-ovule ratio in the selected taxa reflected the breeding system in action. In species showing facultative autogamy, the P/O ratio was very low (266.5 in *Murdannia nudiflora*) when compared to that showing, facultative xenogamy (1508 in *Commelina diffusa*) which in turn was lower than those with predominant xenogamy (2049.33 in *Rhopalephora scaberrima* and 3144 in *Dictyospermum montanum*). The flowers in different orders of *Commelina diffusa* were found to show both facultative autogamy and facultative xenogamy.

Pollen grains showed best germination percentages and pollen tube length in Brewbaker & Kwack medium. Germination in individual inorganic nutrients including calcium nitrate, potassium nitrate, boric acid and magnesium nitrate were only incidental. Presence of esterases and peroxidases in the stigmatic surfaces indicated that the stigma of all studied species became receptive soon after anthesis. The windows of maximum viability periods for the pollen grains and the maximum receptivity periods of the stigma in all species coincided and ensured effective pollen germination in the short period of flower longevity. Pollen and stigma morphology revealed the size, shape, characteristic features of aperture and surface ornamentation, of the pollen grains and the abundance and nature of the stigma surface papillae. These findings could be used for further research in the areas of pollen pistil interactions.

In these plants autogamous pollination acted as a mechanism to ensure pollination incase crossing failed. The facultative cleistogamous flowers produced in response to environmental conditions in *Murdannia nudiflora* and the coiling of the styles in *Commelina diffusa* to facilitate delayed selfing, are mechanisms that ensure seed set and thereby reproduction in these species. *Murdannia nudiflora* showed an increased production of cleistogamous flowers on days with minimal pollinator activity while on days with optimal conditions for pollinators they produced mostly chasmogamous flowers. The presence of andromonoecy in *Commelina diffusa* and the occurrence (though rare) of male flowers in all the other selected species may be indicative of developing mechanisms for enhancing cross pollination.

The major visitors to these species were hymenopterans and dipterans. Even with a lack of nectar as a reward these flowers managed to attract a considerable number of insects with their floral display and the offer of pollen. Most of the visitors were foragers or pollen robbers but occasional predators such as *Lilioceris merdigera* and *Anthocoris* sp. were found on some of the selected species. *Tretragonula iridipennis, Amegill*a sp., *Ceratina*

sp., *Apis* sp., *Halictus* sp., etc., were amongst the most common pollinators. An array of different syrphid flies was also found foraging and effecting pollination in these taxa. *Tretragonula iridipennis* is the most effecive pollinator of both *Dictyospermum montanum* and *Rhopalephora scaberrima,* while *Halictus* sp. is the most effective pollinator for *Commelina diffusa* and *Ceratina* sp. is the most effective pollinator of *Murdannia nudiflora. Apis dorsata, Halictus* sp. and *Amegilla zonata* were all identified as equally effective pollinators of *Floscopa scandens*.

These insects were actively working away on the flowers for the most part of the longevity period. The flower orientation along with the positioning of the dimorphic stamens (except in *Murdannia nudiflora*) within the flowers ensured that the insects got dusted with the pollen grains from the pollinating anthers while offering less or non-viable pollen (from the feeding anthers) for their nourishment, thereby reducing the loss of potential genetic material.

Breeding system studies in the selected taxa revealed that they were all self and cross-compatible species. Except *Dictyospermum montanum* (2.5%) and *Floscopa scandens* (14%), all others showed high percentages of autogamous fruit set (72.38% in *Commelina diffusa,* 47.62% in *Murdannia nudiflora* and 41.43% in *Rhopalephora scaberrima*). *Dictyospermum montanum* seems to favour cross-pollination with a high percentage of fruit set in manually cross-pollinated flowers (76.75%) and slightly lower percentage in manually self-pollinated flowers (50.25%). Similarly *Floscopa scandens* also showed a higher percentage of fruit set when cross-pollinated (84%) and a comparatively lesser percentage when selfed (70%).

Seed germination in the field conditions as well as nursery conditions were observed for all species. While *Dictyospermum montanum* and *Floscopa scandens* failed to germinate in-vitro all the other species showed successful germination. A detailed study into the factors affecting seed dormancy in these species and others of the family presents scope for future investigations.

CONCLUSION

The study on the pollination biology of selected taxa of the family Commelinaceae has given us insights into the phenology, floral biology, pollination biology, and breeding system of *Commelina diffusa*, *Dictyospermum montanum, Floscopa scandens, Murdannia nudiflora* and *Rhopalephora scaberrima* in particular and the family in general. The family Commelinaceae depends on autogamous or entomophilous reproduction. The short flower longevity and a lack of nectar are two factors commonly known to affect the reproductive efficiency in this predominantly entomophilous group of plants.

These entomophilous plants recruit the aid of insects to proliferate, by establishing either a mutual or deceptive relationship with them. The lack of nectar, an important reward for pollinators has seemingly driven these taxa in evolving adaptive features attracting pollinators. The hairy filaments in *Murdannia nudiflora*, hairy sepals in *Floscopa scandens*, staminodes in *Commelina diffusa* and *Rhopalephora scaberrima*, broad connectives**,** coloured anthers, etc., are some such adaptive features found in the studied taxa. Apart from these, division of labour within the androecium in the form of pollinating and feeding stamens (heteranthery), delayed selfing, facultative cleistogamy, andromonoecy, etc., were also found in the studied species. These characters together seem to enhance reproductive efficiency and thereby their survival potential.

These species thus have evolved a system of adaptive modifications which resulted in a mixed mating system that produces both self and cross seeds. Along with this vegetative reproduction ensures the local spread of the population and enhance survival probabilities by increasing the population size.

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Veena V, Nampy S. 2019. Induced cleistogamy: A strategy for reproductive assurance in *Murdannia nudiflora* (Commelinaceae). *Botany* 97(10): 547–557.

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APPENDIX- I

LIST OF PUBLICATIONS

- Veena V, Nampy S. 2019. Induced cleistogamy: A strategy for reproductive assurance in *Murdannia nudiflora* (Commelinaceae). *Botany* 97(10): 547–557.
- Veena V, Nampy S. 2020. Heteromorphic stamen: a strategy in netarless entomophilous plants to increase pollination efficiency? An investigation with regard to three species of Commelinceae. *Plant Systematics and Evolution* 306: 82.

APPENDIX- II

PAPERS PRESENTED IN INTERNATIONAL/NATIONAL SYMPOSIA/WORKSHOPS

1. **Veena V.** & Nampy, S. **2015.** Pollen Viability and Receptivity in *Dictyospermum montanum* (Commelinaceae). Silver Jubilee Conference of IAAT and Council Meeting of IAPT & International Seminar on Advances in angiosperm systematics and conservation held at University of Calicut, Kerala, India. November, 2015.

2. **Veena V.** & Nampy, S. **2016.** Possible significance of floral organ structural variations in *Dictyospermum montanum* Wight (Commelinaceae). XXVI Annual Conference of Indian Association for Angiosperm Taxonomy and International Seminar on Conservation and Sustainable Utilization of Biodiversity held at Shivaji University, Kolhapur. November, 2016.

3. **Veena V.** & Nampy, S. **2017.** Anther dimorphism and probable consequences in three species of the family Commelinaceae. XXVII Annual conference of Indian Association for Angiosperm Taxonomy and International symposium on "Plant Systematics: Priorities and Challenges organized by University of Delhi. November, 2017.

4. **Veena V.** & Nampy, S. **2018.** Pollen dispersal, pollen viability and pistil receptivity in *Murdannia nudiflora* (Commelinaceae). XLI All India Botanical Conference of The Indian Botanical Society and "Ecological Restoration, Carbon Sequesteration and Biotechnological Approaches for Biodiversity Conservation", held at Jiwaji Univrsity, Gwalior. October, 2018.

5. **Veena V.** & Nampy, S. **2019.** Andromonoecy and the breeding system of *Commelina diffusa* Burm. f. XLII All India Botanical Conference of The Indian Botanical Society And national symposium on "Innovations and inventions in plant science research", held at the University of Calicut, Malappuram. November, 2019.