MELISSOPALYNOLOGICAL STUDIES OF STINGLESS BEE *Tetragonula travancorica* (APIDAE: MELIPONINI)

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(2017-11-142)

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COLLEGE OF AGRICULTURE

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KERALA, INDIA

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by

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(2017-11-142)

THESIS

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2020

DECLARATION

I, hereby declare that this thesis entitled **"Melissopalynological studies on stingless bee *Tetragonula travancorica* (Apidae: Meliponini)"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Melissopalynological studies on stingless bee *Tetragonula travancorica* (Apidae: Meliponini)"** is a record of research work done independently by Ms. Lincy Abraham (2017-11-142) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

@	At the rate of
µm	Micrometre
P	Polar length
E	Equatorial diameter
l	Length
b	Breadth
DNA	Deoxyribo nucleic acid
MQ	Milli-Q
PCR	Polymerase Chain Reaction
rpm	Rotations per minute
et al.	and other Co workers
Fig.	Figure
g	Gram
i.e.	That is
KAU	Kerala Agricultural University
viz.	Namely
° C	Degree Celsius
µL	Microlitre
mL	Millilitre
mg	Milligram
ng	Nanogram
mm	Millimetre
min	Minutes
Temp.	Temperature
Conc.	Concentrated
h	Hours
m	metre
P. type	Pollen type

bp	Base pairs
cpDNA	Chloroplast DNA
w/v	Weight/ Volume

Introduction

1. INTRODUCTION

Stingless bees are considerably the biggest group of eusocial bees on Earth and they belong to the super family Apoidea, family Apidae and sub family Meliponinae. Meliponinae consists of eight genera and 15 sub-genera (Wille, 1983) out of which two important genera are *Melipona* and *Trigona* belonging to the tribes Meliponini and Trigonini, respectively. All Asian and African species of stingless bees belong to the tribe Trigonini (Camargo *et al.*, 1988). With over five hundred represented species, stingless bee outdo the honey bees by an element of fifty and even comprise double the quantity of known bumble bee species (Camargo and Pedro, 2007; Michener, 2007). The genus *Tetragonula* Moure which is a complex genus with more than 30 species described is the most widespread in Srilankan and Indian subcontinent (Rasmussen, 2013). *Tetragonula travancorica* Shanas and Faseeh is the new species under the subgenus *Tetragonula* which is often confused with *T. iridipennis* Smith. This species is distributed throughout the southern part of India and can be well distinguished from other species in India by the presence of strongly nebulous radial vein on hind wings and dark brown erect setae on margin of mesoscutellum (Shanas and Faseeh, 2019).

Stingless bees have accomplished the supreme level of social organization which is similar to that of the honey bees (Sakagami, 1982). They are found in tropical and southern subtropical areas all through the world which live in perpetual colonies of a few hundred to several thousand workers with most of species settling in preformed cavities in live trees, in old walls, crevices and such other concealed places (Michener, 2013). They are called stingless bees since they cannot sting due to the absence of venom apparatus, apparently with a small, reduced vestigial sting without an effective tip. To defend their colonies they chase and bite the intruders causing annoyance by crawling into the nose, eyes and ears and entangling in hair.

They collect dammer or the resin for construction purpose which is then mixed with wax produced from their body to produce cerumen, the building material. In addition to collecting resin, they also collect mud and saw dust for their

nest substances. Besides, some of the species has been believed to gather salt and water from the dead animal body and urine (Baumgartner and Roubik, 1989). Stingless bees are usually polylectic, opportunistic foragers on several of plant species that provide them pollen and nectar (Wilms *et al*., 1996) which comprises their main food sources (Wilson and Carril, 2015).

Pollen is the protein-rich food that plays a pivotal role in the growth of the brood to retain colony strength and reproduction whereas nectar provides the energy for flight, foraging, and other hive activity (Grogan and Hunt, 1979; Boumgartner and Roubik, 1989; Willis and Kevan, 1995). Stingless bees respond well to flowering and can change habit from solitary foraging to group foraging in presence of a good food source. All of them are generalist plant and flower visitors (Abrol, 2011), and some species are known to visit and utilize floral resources from more than a hundred plant taxa through the span of a few seasons in its habitat.

Pollen and honey are stored in specialized cells or pots built of cerumen. The brood comb is seen separate from honey and pollen pots and the latter are bigger in size. They dump waste in piles which contains dead bees and their parts, remains of brood, cocoons as well as faecal matter (Devanesan *et al*., 2012).

Information on the foraging activity and other attributes of stingless bees is of much use for meliponiculture (rearing of stingless bees) and in management of stingless bees and thus better to be utilized in planned pollination also (Singh *et al*., 2015). It is a necessity to realize the active time of foraging of bees so as to preserve the bee species and to sustain the vital ecosystem service of pollination as well as the biodiversity associated with them.

Foraging activity in bees is a routine and an essentiality for their survival, although less foraging activity is seen during cloudy or rainy days. The pattern of activity in a colony is the conduct of individual honey bees and the information exchange between the workers is the key to colony foraging efficiency. Foragers are influenced by accessibility of food and its quality, climate, rivalry and behaviour of returning successful nestmates (de Bruijn and Sommeijer, 1997). The immediate

requirement of colony, internal conditions such as amount of honey, pollen or resin stored also dependably stimulates the stingless bee forager and affects the flight activity.

Individual foraging choices are not only directed by colony requirements, but also by prior experience/ individual memory through feedback mechanisms (Biesmeijer and Toth, 1998). The extrinsic information sources provide an incessant stream of information to the forager who incorporates it with its intrinsic information to create behavioural decisions (Biesmeijer and Slaa, 2004). Also the colony development and growth rely upon the amount and nature of nectar and pollen collected by bees (Mohapatra *et al.,* 2014).

The bee and plant relationship is very significant in an ecosystem which in a way profit both of them and allowing them to withstand. Identification of plants visited by stingless bees for pollen and nectar and their bloom pattern is useful in the selection of sites suited for meliponiculture in the region. Melissopalynology is the study of the pollen they collect, or which gets into honey intentionally and accidentally. This least expensive and quickest way to determine the floral contents and geographical origin of honey is a compelling method for understanding the collaboration between honey bees and vegetation, and is significant in building up apiculture-based honey industries. An assessment of plants for their utility as bee forage sources provides enough information needed to measure the potential for beekeeping in an area (Jhansi *et al.*, 1991). The collection of pollen by bees also provides valuable pollination services for many plants (Nabhan and Buchmann, 1997).

Melissopalynological studies concerns with microscopic analysis of the pollen contents of seasonal honeys and also pollen loads from a locality. This analysis when enhanced with critical field studies involving the phenology and floral biology gives predictable data regarding the floral types which serve as major and minor nectar and pollen sources for the honey bees (Attri 2010). Information from pollen analysis mirrors the floral situation of the place where specific honey

was produced and indicates the geographical origin based on the presence of a combination of pollen types (Sivaram *et al*., 2012). The vegetation pattern and plant species peculiarly influence the activities of bee colonies and overall honey production (Rimna *et al.*, 2017).

Melissopalynological studies are thus helpful in bee management and in promoting apiculture. The analysis of the pollen in the honey and pollen loads or stores have proven to be a useful tool in assessing bee diet and are essential to identify the geographical and botanical origin of honey. However, our insight on foraging activity, nectar and pollen sources of stingless bees from Kerala particularly Thiruvananthapuram district is inadequate. For the best utilization of *Tetragonula travancorica* Shanas and Faseeh for honey production and pollination, this information is significant. Hence, to connect these examination holes, the present investigation "Melissopalynological studies on stingless bee *Tetragonula travancorica* (Apidae: Meliponini)" were made with the accompanying objectives:

1. To study the foraging activity of *T. travancorica* in three different seasons.
2. To carry out melissopalynological studies of honey samples and pollen loads of *T. travancorica* and identify its floral resources from different locations in Thiruvananthapuram district.

Review of Literature

2. REVIEW OF LITERATURE

Honey and beekeeping have a long history in India. Honey was the first sweet food tasted by the ancient Indians inhabiting rock shelters and forests. Both the natural and cultivated vegetation in India constitute an immense potential for development of beekeeping. About 500 flowering plant species, both wild and cultivated, are useful as major or minor sources of nectar and pollen.

Among the honey bees, stingless bees are the important pollinators in tropical and subtropical parts of the world. They differ from *Apis* sp. taxonomically, as they come under the subfamily Meliponinae of Apidae family. The subfamily consist of two tribes Trigonini and Meliponini (Wille, 1979). All of the Asian species belongs to the genus *Tetragonula* Moure under tribe Trigonini (Danaraddi and Viraktamath, 2009; Michener, 2013).

Tetragonula iridipennis Smith was the first described type species of genus *Tetragonula* in India by Smith (1954) in Srilanka, later on from India. The widespread presence of *T. travancorica* Shanas and Faseeh from the peninsular region of South India was reported by Shanas and Faseeh (2019) for the first time. The literature pertaining to the foraging, hive activity and melissopalynological studies conducted so far has been reviewed in this chapter.

2.1 MELIPONICULTURE

Meliponiculture is the scientific keeping and management of stingless bees. It is practised as backyard beekeeping mainly for honey production where stingless bees are kept either in bamboo nodes or mud pots or box hives (Muthuraman and Saravanan, 2004). Stingless bees naturally occur in cavities, tree trunks or old walls or inside the termite mound or subterranean cavities. They also exhibit habitats to build their nest around human dwelling spaces by using human-constructed materials for nesting (Karthick *et al*., 2018).

In the New World, Native Americans in Mexico and Central America developed beekeeping utilizing an assortment of stingless bees where they could

get around two kg of honey from one stingless bee colony. Today, the United States is a major honey producer but ranks far behind China and Turkey (Bryant, 2018).

Bee-keeping involving several species of native honey bees (*Apis* spp.) is a very important enterprise with a long tradition in the Indian subcontinent less known is the fact that stingless bees have also been kept for centuries in India, Sri Lanka and Nepal (Crane 1999, Kumar *et al*., 2012).

In some parts of Kerala, hiving is done in coconut shells, which makes it easier to split colonies and extract honey (Muthuraman and Saravanan, 2004). The Kani tribes living in Karayar area of Kalakad- Mundanthurai Tiger Reserve area (KMTR) of Western Ghats have been practising Meliponiculture for years using hollow trunks of bamboo to domesticate the feral colonies inside which, bees created three chambers *viz.*, honey storage chamber, pollen storage chamber and brood-rearing chamber (Kumar *et al*., 2012).

Hiving the feral colonies was the only source for acquiring the colonies for beekeeping. The potential of stingless bees for crop pollination is enhanced by the ability to transfer colonies into an artificial hive (Heard, 1999).

2.2 PROSPECTS OF MELIPONICULTURE

Stingless bees are regular visitors of crop plants in tropical region. More than 1000 plant species are cultivated in the tropics for food, beverages, fibre, spices, and medicines, approximately 250 species adapted to pollination by stingless bees (Heard, 1999) and are known to be an important pollinator in tropical rainforest (Eltz *et al.*, 2003). Stingless bees have a short flight range, and being good candidate, their importance is realised providing pollination services in several agricultural ecosystems and green house (Slaa *et al.*, 2006) and of various local and cultivated tropical plants (Makkar *et al*., 2018).

Meliponiculture is apt for women because it does not involve hefty physical labor and provides an added income for the rural families (Devanesan *et al*., 2009). Stingless bee colonies live for a long time and do not pose environmental risks.

Stingless bee hives can be transported wherever needed for pollination and propagated so that growers do not have to depend on natural populations (Karthick *et al.*, 2018). They are active over a wide range of climatic conditions and if conditions become unsuitable, the colonies can be moved to better area.

Beekeeping with stingless bees, *i.e*., Meliponiculture has been realized to be profiting both stingless bees and indigenous communities. Most species do not irritate man and they can be controlled securely and supervised effectively in the homesteads. Meliponiculture can be a feasible marginal occupation for people in rural area, especially women and youth (Chidi and Odo, 2017; Palial *et al*., 2019), since its cost and efforts required are less. Honey obtained from colonies in forests have high medicinal properties, mainly used for ayurvedic preparations also fetch higher price for the farmers. Honey derived from stingless bees are of high quality having therapeutic effects (Rahman *et al.*, 2013).

Choudhari *et al.* (2012) reported 24 chemical compounds as identified from the Indian stingless bee propolis out of which, 15 compounds are being reported for the first time in Indian stingless bee propolis having anti-microbial properties.

Meliponiculture is less advanced than apiculture in India (Muthuraman and Saravanan, 2004). Meliponiculture is yet to develop in India as its scope is not fully identified and exploited. Intensive research on biodiversity, hives design standardization, colony management, honey extraction techniques, biology, foraging activities and sources, usage and marketing of hive products have to be done to expand and popularize meliponiculture in India.

2.3 MELISSOPALYNOLOGY

Melissopalynology is the branch of palynology that reviews pollen and spores in honey (Ebenezer and Olugbenga, 2010). The effectiveness of the strategy relies upon the abilities of the pollen analysis, the technique for extracting the pollen from honey samples and the expertise of the analyst in interpreting the results.

Melissopalynology deals with the qualitative and quantitative analysis of honey palynoflora which indicates nectar and pollen sources of honey bees in a specific geographical area in particular seasons. Using proper techniques pollen grains of flower species may be studied to determine the geographical origin and major floral source of the honey. It has been broadly used to decide the purity, geographical and botanical origins of honey. Pollen grains are acquired directly from the honey bee body or taken from its nests and colonies (Peterson and Bryant, 2011). The rich and diverse botanical resources, warm and favourable climate of region are essential elements for beekeeping. Knowledge about the plants utilized as resources by the bees is an additional benefit towards the conservation and continuance of species important to produce honey (Absy *et al.*, 2018).

Pollen analysis plays a crucial role in supporting the legitimate business of marketing honey with the confidence that it is suitably designated and in discouraging mislabelling where this happens (Martin, 2005). Melissopalynology still has a promising future as an approach to distinguish the floral sources, possibilities of contamination, probability of blending and the determination of local or foreign origin of a sample (Bryant, 2018). Melissopalynology currently remains the most economical and quickest strategy to find reliable solutions, provided the pollen extraction and investigation is conducted by experienced personalities.

2.3.1 METHODS OF MELISSOPALYNOLOGY

Erdtman's (1960) acetolysis technique is the most popular method followed worldwide. Different methods of honey analysis with and without acetolysis was proposed by Louveaux *et al.* (1970). Although, later International Commission for Bee Botany (ICBB) in 1978 elaborated, proposed and published a standard method of melissopalynology which included acetolysis procedure. Lieux (1980) formulated a combined method which allows collection of quantitative and qualitative data in one procedure. Harmonized methods for qualitative and

quantitative analyses along with the results of the ring trials for repeatability of acetolysis was published by Von Der Ohe *et al*., 2004).

Acetolysis enables more accurate pollen identification as it dissolves most of the tissue, organic debris and biomolecules from the surface of the pollen grains particularly exine, which possess diagnostic characteristics are easily seen (Erdtman 1963; Low *et al*. 1989). Additionally, the pollen grains can be photographed in both the equatorial and polar views.

Jones (2014) provided a detailed, step by step acetolysis technique that recovers pollen from any pollinator and described the acetolysis technique along with the necessary precautions.

2.3.2 MELISSOPALYNOLOGICAL STUDIES

Ramalho *et al.* (1994) reported that 97 per cent of stingless bee foragers visited only one floral source on each trip and transported unifloral pollen loads of *Eucalyptus* spp., followed by *Archontophoenix cunninghamiana* Wendl. et Drude, *L. leucocephala*, *Alchornea sidaefolia* Baill, *Dombeya wallichii* Daydon Jackson and 7 additional pollen types. Other important pollen were *Croton floribundus* Spreng., *Piptadenia gonoacantha* (Mart.) J. F. Macbr., *Eugenia uniflora* L., *Tipuana tipu* (Benth.) Kuntze, *Mimosa velloziana* Mart., *Impatiens sultanii* Hook. f., *Schizolobium parahyba* (Vell.)S.F.Blake and *Cecropia pachystachya* Trecul.

Faria *et al*. (2012) identified 86 pollen types belonging to 66 genera under 36 families in the samples. The families with the highest number of visited species were Fabaceae, Malvaceae and Myrtaceae. Thirty families and 54 genera of plants were identified from 66 pollen types collected from foraging bees of *Scaptotrigona* aff. *depilis* Moure. Trees were broadly exploited representing 68 per cent of the plants used by the bees, especially *Eucalyptus moluccana* Wall. ex Roxb., *E. grandis* W.Hill, and *Myracrodruon urundeuva* Allemao were intensively exploited (Aleixo *et al*., 2017).

Iwama and Melhem (1979) reported *S. terebenthifolius, Ambrosia* sp., *Alchornea triplinervia* (Spreng.) Müll.Arg., *Euphorbia splendens* Bojer ex Hook., *Eucalyptus cinerea* F.Muell. ex Benth., *E. robusta* Sm., *E. rudis* Endl., *E. tereticornis* Sm., *Trema micrantha* (L.) Blume, *Mikania* sp., *Phyllanthus* sp., *Leucaena glauca* Benth., *Piptadenia rigida* Benth., *Sorocea bonplandii* (Baill.) W.C.Burger, Lanj. & de Boer, *Eucalyptus* sp., *Jasminum azoricum* L., and *Petroselinum hortense* Hoffm. to be the predominant plant species in the honey from two colonies of *Tetragonisca angustula angustula* Latreille.

Analysis of pollen and honey samples taken from *Melipona marginata marginata* Lepeletier showed a total of 173 pollen types, belonging to 32 different plant families, of which 105 were used to collect pollen and 124 to collect nectar. Most species represented in samples were from Leguminosae, followed by Compositae, Euphorbiaceae and Myrtaceae and though Myrtaceae was most visited for both pollen and nectar (Kleinert-Giovannini and Imperatriz-Fonseca, 1987).

Six species, *Trigona (Homotrigona) fimbriata, T. (Odontotrigona) haematoptera* Cockerell, *T. (Tetragonula) collina, T. (Tetragonula) laeviceps, T. (Tetragonula) melanocephala,* and *T. (Tetrigona) binghami* Schwarz were observed. *Archidendron jiringa* (Jack) I.C.Nielsen, *Ardisia elliptica* Thunb., *Cassia fistula* L., *P. pterocarpum, Dillenia excelsa* (Jack) Martelli ex Gilg., *Mallotus* sp., *Ixora javanica* (Blume) DC. and *Wedelia trilobata* (L.) Hitchc. were found to be good sources for bees (Leonhardt *et al*., 2007).

Luz *et al.* (2011) melissopalynologically analysed 33 pollen types collected from *Melipona capixaba* Moure and Camargo hives and identified 23 genera from 15 families. The families Fabaceae showed the highest richness of pollen types followed by Myrtaceae, Solanaceae, Arecaceae, Asteraceae, Euphorbiaceae, Melastomataceae/Combretaceae, Rubiaceae, and Sapindaceae. *Eucalyptus* and *Tibouchina* were important sources of pollen.

Pollen loads collected from the corbiculae of *T. angustula* were analysed and the results showed 18 pollen types belonging to 16 plant families. Frequently

visited families includes Melastomataceae, Myrtaceae, Piperaceae, Caesalpiniaceae, Meliaceae, Cyperaceae and Cecropiaceae. The important pollen identified were *Schizolobium parahyba* (Vell.) S.F.Blake, *Cyperus* sp., *Tetrapterys* sp., *Tibouchina granulosa* (Desr.) Cogn., *Trichilia* sp., *Anadenanthera colubrina* (Vell.) Brenan, *Eucalyptus* sp., *Passiflora jilekii* Wawra, *Piper mollicomum* (Kunth) Steud. and *Cecropia hololeuca* Miq. (Morgado *et al*., 2011).

A total of 306 pollen types were identified which belonged to 49 families by Obregon *et al*. (2013) from honey samples of *T. angustula* colonies in Colombia. The higher representations of pollen in samples was of family Asteraceae (49), then Fabaceae (39), Malvaceae (11), Rubiaceae (11), Melastomaceae (11), and Euphorbiaceae (11). The most frequent pollen was *Heliocarpus americanus* L., *Coffea arabica* L., *Citrus* sp. and *Myrcia* type.

Ramalho (1990) reported that even though 92 plant species appeared in the samples of *Scaptotrigona* spp. only 6 sources were heavily exploited. *Eucalyptus* spp, *Myrciaria cauliflora* were predominant sources while *P. gonoacantha*, *M. daleoides*, *Sambucus australis* Cham. & Schltdl. were secondary pollens. Myrtaceae and Leguminosae were the families highly represented in the diet.

Ramalho *et al*. (1990) on comparative study between stingless bees and africanized honeybees found *Alchornea* sp., *Baccharis* sp., *Cassia* sp., *Cecropia* sp., *Croton* sp., *Euphorbia* sp., *Miconia* sp., *Mimosa* sp., *Piptadenia* sp., *Solanum* sp., *Tibouchina* sp., *Trema* sp. and *Vernonia* sp. to be important. Out of a total 288 plant species, 126 and 52 plant species were important for Trigonini and *Melipona* respectively. Fifty three plant species were found important for both Trigonini and Melipona. The families Anacardiaceae, Compositae, Euphorbiaceae, Labiatae, Leguminosae, Melastomataceae, Moraceae, Myrtaceae, Palmae, Rubiaceae and Solanaceae constituted the most consistent sources of pollen and nectar.

In a study conducted by Rech and Absy (2011a), 104 pots of pollen of *Trigona*, *Partamona* and *Scaura* spp. revealed 78 pollen types, 47 of those being identified at species level belonging to 36 botanical families. The nine most

abundant families found were Urticaceae, Arecaceae, Fabaceae, Euphorbiaceae, Simaroubaceae, Moraceae, Verbenaceae, Malpighiaceae, and Lamiaceae. The most frequent pollen type was Myrtaceae.

Studies on the diversity of pollen sources and niche overlap of three species of stingless bees, *T. collina*, *Scaptotrigona pectoralis* Dalla Torre and *T. angustula* were conducted. Analysis revealed *Moraceae* aff., *Bursera simaruba* (L.) Sarg., *Inga* sp., *Sapindus saponaria* L., and members of Rutaceae, Mimosaceae and Rubiaceae families were important (Sanchez- Chaves and Van Nieuwstadt, 1996).

Sommejier *et al.* (1983) collected and analysed samples from *Melipona scutellaris* Latreille and *M. favosa* which contained 44 pollen types in which 20 types could be identified upto species and 4 to genus. *Spondias mombin* L., *Delonix regia* (Bojer) Raf., *Mimosa* sp., *C. nucifera*, *P. guajava*, Solanum sp. were found to be dominant sources. *P. guajava* was an important source throughout the observation period.

Pollen spectrum was obtained for honey samples taken from the nests of 48 *Melipona* spp. and 20 other stingless bee species in Venezuela. The abundant pollen types in honey were *M. pudica*, *Machaerium* sp., *Avicennia* sp., *Mimosa scabrella* Benth., *Cassia* sp., *Myrcia* sp., *Piper* sp., *Philoxerus* sp., *Xanthoxylum* sp., *Alternanthera* sp. and *Astragalus* sp. (Vit and Ricciardelli d'Albore, 1994).

Stingless bees visited single floral resources at each trip, which is evidenced by the pure pollen loads in their corbiculae. In Brazil, 97 per cent of pollen foragers of nine species of stingless bees visited only one floral resource (Heard, 1988). Vossler *et al*. (2010) analysed 146 honey pots and 63 pollen pots and identified pollen from 39 different taxa belonging to 30 families in pollen samples. *Prosopis* spp., *Ziziphus mistol*, *Capparis* spp. and *Maytenus*-type were predominant type of pollen whereas *Castela coccinea* and *Pisonia zapallo* were also important sources. All these six taxa were also recorded as important nectar sources.

Balderas (2016) collected pollen samples from the pollen pots and the corbiculae of the returning foragers of *Tetragonula biroi* Friese in upland and lowland ecosystems of Philippines. Eighty four plant species under 43 families were identified out of which 16 per cent were secondary to predominant and 84 per cent were minor or important minor species. The predominant pollen were of *C. nucifera, Ceiba pentandra* (L.) Gaertn., *Syzygium samarangensi* (Bl.) Merr & Perry, *Pterocarpus indicus* Willd, *Elaeis guineensis* Jacq. and *Mangifera indica* L. *C. nucifera* was pollen source in all sites. *C. pentandra, M. indica, E. guineensis, P. indicus, Syzygium* spp., *Persea Americana* Mill., *Areca catechu* L. and *Citrus* spp. were dominant to secondary pollen sources in the lowland ecosystem. On the other hand, *Citrus* spp. and *Muntingia calabura* L. were the dominant pollen sources in the upland ecosystem.

Study conducted in an apiary of Besut, in the Terengganu state concluded that, a total of 11 pollen types were collected by *L. terminata* foragers. The most frequently collected pollen was from *Ixora coccinea* L. (36.78 per cent), followed by *Citrus hystrix* DC. and *Murraya paniculata* (L.) Jacq. all the three of which were considered accessory pollen. While isolated pollens consist of *Mimosa pudica* L., *Callophyllum inophyllum* L., *Asystasia gangetica* L. and *Bougainvillea glabra* Choisy (Type 1). The highest percentage of pollen was from *C. hystrix*, *C. inophyllum* and *I. coccinea* in November, January and February respectively (Azmi *et. al.,* 2015).

Pollen load were collected from worker of two species of stingless bee *Tetragonula pagdeni* Schwarz (8 colonies) and *Lepidotrigona terminata* Smith (4 colonies). Pollen of 17 plant species was found in pollen load in which most common were *P. pterocarpum* and *Z. mays* (Sawatthum and Kumlert, 2015).

In a total of 307 pollen loads collected from 3 colonies (*T. collina, T. melanocephala* Gribodo and *T. melina* Gribodo), 74 pollen types were distinguished of which 46 were identified as belonging to 18 plant families. The most abundant pollen types belong to family Araceae, followed by Annonaceae and

Euphorbiaceae. Only one species of Euphorbiaceae occurred in 3 colonies (Nagamitsu and Inoue, 2002).

Pangestika *et al*. (2017) reported flower constancy of *T. laeviceps* on Poaceae (76.49 per cent) followed by member of Rutaceae family, *L. terminata* on Euphorbiaceae (80.46 per cent) followed by member of Arecaceae and *H. itama* on Solanaceae (83.33 per cent) followed by another member of Arecaceae in a work carried out in Indonesia.

Nagamitsu *et al*. (1999) collected the pollen samples from 693 returning foragers and 102 pollen types were identified and 55 upto family level. The most abundant family was Euphorbiaceae followed by Araceae, Annonaceae and Leguminaceae.

Jongjitvimol and Wattanachaiyingcharoen (2006) found 29 plant species under 23 genera and 18 families in pollen loads collected by three species of the stingless bees; *Trigona (Tetrigona) apicalis* Smith, *T. collina* and *Trigona (Homotrigona) fimbriata* Smith. The main food sources of *T. apicalis* were *Tridax procumbens* L. (Asteraceae), *Mimosa pigra* L. and *M. pudica* (Mimosaceae) and *Tectona grandis* L. f. (Verbenaceae). The main food sources of *T. collina* were *T. procumbens* (Asteraceae), *Fernandoa adenophylla* (Wall. ex G.Don) Steenis (Bignoniaceae), *M. pigra* and *M. pudica* (Mimosaceae) and *T. grandis* (Verbenaceae). Plant species of two families, *Lagerstroemia calyculata* Kurz, *L. macrocarpa* Wall. and *L. tomentosa* C. Presl (Lythraceae) and *M. pigra* and *M. pudica* (Mimosaceae) are the main food sources for *T. fimbriata*. All *Trigona* species collected pollen from 9 plants namely *R. tuberose*, *T. procumbens*, *F. adenophylla*, *Senna siamea* (Lam.) H.S.Irwin & Barneby, *Ipomoea aquatica* Forssk., *Merremia vitifolia* (Burm. f.) Hallier f., *M. pigra*, *M. pudica* and *Erythrina stricta* Roxb. *Caryota bacsonensis* Magalon and *Dalbergia lanceolaria* L.f. were found to be specific food plants of *T. collina.*

Melissopalynlogical analysis revealed a total of 45 pollen types belonging to 22 families in 72 honey samples of *T. pagdeni* collected from six locations in

Thailand. The predominant pollen type in two of the locations was *Nephelium lappaceum* L. (Sapindaceae), whereas in two other locations it was classified as a secondary pollen type. *Wodyetia bifurcate* A.K.Irvine (Arecaceae) was especially prevalent in one location as secondary pollen. *C. nucifera* (Arecaceae) and *M. pudica* (Leguminosae) were also abundant sources in honey samples at all locations. *A. gangetica, Amaranthus lividus* L., *A. catechu*, *Chromolaena odorata* (L.) R.M.King & H.Rob. and *Durio zibethinus* L. were found in all honey samples (Thakodee *et al*., 2018).

Eltz *et al*. (2002) investigated *Trigona collina* Smith garbage in Sabah, Malaysia and reported a total of 148 pollen from 38 families. *Rhizophora apiculata* Bl. was predominant in the garbage of *T. collina* and other 3 species, signifying it as an important resource for most species. Pollen diets were dominated by plants like *R. apiculata, Z. mays*, *Manihot esculenta* Crantz. and *Citrullus lanatus* (Thunb.) Matsumara & Nakai and contained minor sources like *M. pudica, M. esculenta* and ornamental plant *Turnera ulmifolia* L.

Rosdi *et al*. (2016) conducted Melissopalynological studies in North Malaysia which revealed Fabaceae (*Albizia falcataria* (L.) Fosberg, *M. pudica*, *M. caesalpiniifolia* Benth., *M. scabrella*, *Sophora japonica* L.) to be most frequent and highly represented family out of 12 families observed. This was followed by Myrtaceae and Arecaceae.

Ramanujam *et al.* (1993) analysed the pollen from honey samples collected from a colony of *T. iridipennis* in Hyderabad, India and found to be of unifloral origin with *Prosopis juliflora* (Sw.) DC as the predominant pollen type and *Rotala densiflora* (Roemer and Schultes) Koehne, *Peltophorum pterocarpum* (DC.) K. Heyne and *Cocos nucifera* L. as important secondary pollen types. *Eucalyptus globulus* Labill., *Cyanotis Sp, Leucaena leucocephala* (Lam.) de Wit, *Tamarindus indica* L., *Delonix regia* (Boj. ex Hook.) Rafin., *Azadirachta indica* A. Juss, *Lawsonia alba* Lam. nom. illeg., *Ageratum conyzoides* L. *and Loranthus longiflorus* Desr. are constituted minor pollen types. They also analysed 430 pollen

loads collected from *T. iridipennis*, of which 367 were unifloral, 60 bifloral and only 3 were multifloral.

Danaraddi (2007) analysed 36 honey samples and 180 pollen loads taken from incoming worker bees from hived colonies during June 2006 to May 2007. It was found that, *Peltophorum ferrugenium* (Decne.) Benth. formed predominant source of nectar throughout the study period *i.e.* from June to May. *C. nucifera* formed the secondary source in June and from July to November besides *C. nucifera, Alternanthera sessalis* L. also formed the secondary source. *Moringa olifera* Lamk served as an additional secondary source for the stingless bees from December to May. He also identified 36 plant species belonging to 24 families from pollen loads which included six field crops, four vegetables, eight fruits and plantation crops, two ornamentals, seven weeds and six tree species and one grass.

Vijayakumar and Jeyaraaj (2016**)** conducted melissopalynological investigation on 15 different honey samples collected from *T. iridipennis* in Nellithurai, Tamil Nadu. Forty five pollen types belonging to 29 families were found to be source for pollen, nectar and resin. The foragers preferred 12 plants species belonging to 4 families namely Asteraceae, Musaceae, Fabaceae and Poaceae. The highest frequency (71 to 100 per cent) was noted in pollen of *C. nucifera, Zea mays* L., *Musa paradisiaca* L. *and Commelina benghalensis* L. while lowest frequency (11 to 20 per cent) was observed for *Vitex negundo* L., *Coriandrum sativum* L., *Tribulus terrestris* L. and *Pennisetum americanum* L. Pollen grains of *C. nucifera, Helianthus annus* L. and *M. paradisiaca* were identified as secondary pollen group.

Five samples of honey were procured from Kannur and Wayanad districts of Kerala, one sample (S1- Cheruthaen) from Wayanad, three samples of *Apis* sp. from Kannur and one from market. Thirty six pollen types were identified out of which 29 pollen types belonging to 15 families were in stingless honey. Stingless bee honey constituted the highest pollen count which was found to be 2074 contributing 58 per cent of the total pollen grains and no predominant types were

observed. The highest contribution was of *Pennisetum polystachyon* L. (33.39 per cent) which was observed as the secondary pollen type and *Acacia auriculiformis* Benth., Acanthaceae type, *Adenanthera pavonina* L., Asteraceae type 1, *Caesalpinia bonduc* L., *Croton bonplandianum* Baill., *Passiflora foetida* L. and Lamiaceae type were represented below 1 per cent. Tree species dominated in sample followed by herbs, shrubs and climbers in the sample (Rimna *et al*., 2017).

Layek and Karmakar (2018) collected 48 honey samples from 48 wild colonies and majority of the honey samples (43) were found to be multifloral in origin. Eighty four plant taxa were identified as nectar sources for the bee species. The predominant pollen types were *Borassus flabellifer* L. (during summer), *Brassica nigra* L. and *E. globulus* (during winter), and *Lannea coromandelica* (Houtt.) Merr. (during spring). Highest number of nectariferous taxa was obtained during summer and monsoon (28 taxa in each season), followed by spring (25 taxa), winter (16 taxa), autumn (12 taxa), and late autumn (9 taxa). From the analysis of 1558 pairs of corbicular pollen loads, 96 plant taxa were identified as sources of pollen loads. Frequently occurred plant taxa were *B. flabellifer* and *Terminalia arjuna* (Roxb.) Wight & Arn. (summer) *A. auriculiformis, E. globulus* and *Ziziphus mauritiana* Lam. (during autumn) *A. auriculiformis* and *E. globulus* (late autumn) *B. nigra* and *Phoenix sylvestris* (L.) Roxb. (winter) and *B. flabellifer* and *L. coromandelica* (spring). During monsoon, pollen of all plant taxa were less frequent. Maximum number of plant taxa were obtained during monsoon (38 taxa), followed by summer and spring (29 taxa in each season), winter (15 taxa), autumn, and late autumn (9 taxa in each season). The family represented by maximum number of plant species was, Fabaceae (17 taxa), followed by Asteraceae (8 taxa), Cucurbitaceae, Euphorbiaceae, Malvaceae, Myrtaceae, Rutaceae (each of them with 5 taxa). Seasonwise highest number of foraged plants for the bee species was obtained during monsoon (nectariferous 28, polleniferous 38, total bee plants 45). Twenty one plant taxa served only as sources of nectar, 33 plant species as pollen source and remaining 63 plant taxa served as sources for both nectar and pollen.

A comparative study was conducted in Pune to compare honey samples of *Trigona* and *Apis* species. Predominant pollen was absent in honey sample collected from *T. iridipennis* which had nine pollen types. *M. oleifera, P. pterocarpum* and *A. conyzoides* were the secondary pollen while the minor pollen types were *Amaranthus* sp., *C. nucifera*, *Bombax ceiba* L., *Sorghum vulgare* (L.) Pers., *Z. mays* and member of Ceasalpiniaceae family. *P. pterocarpum* is a major source of nectar for *A. cerana*, *A. dorsata* and *T. iridipennis*. *A. conyzoides*, *Amaranthus* sp., *C. nucifera* and member from Commelianaceae family were visited by all the bee species (Joshi *et al.*, 1998).

Viraktamath *et al*. (1999) conducted a melissopalynological study in Dharwad on four of *Apis* sp. and one *Trigona* species from December 1996 to March 1997. *Trigona* sp. exploited 17 species of plants with *P. pterocarpum* to be most common source for all the bee species. Other important sources included *Euphorbia pulcherima* Willd. ex Klotzsch, *Eupatorium odoratum* L., *Antigonon leptopus* Hook. & Arn., *Gaillardia* sp., *Petunia* sp., *Bougainvillea* sp. *M. pudica*, *Quisqualis indica* L., *Erythrina* sp., *Bignonia venusta* Ker Gawl., *A. indica*, *Samanea saman* (Jacq.) Merr., *Salvia* sp., and *Bauhinia purp*urea L.

Pollen analysis of pollen loads and honey conducted by Aswini (2013) in *Apis cerana indica* Fab. colonies in southern districts of Kerala revealed presence of 69 pollen types in which 24 were identified up to species level. *C. nucifera* and *M. pudica* were found contributing throughout the year.

Shwetha (2013) identified 45 plant species belonging to 26 families from the honey samples in Kerala. The family that showed the highest frequency was Fabaceae, followed by Euphorbiaceae, Arecaceae, Caesalpinaceae, Convolvulaceae, Cucurbitaceae, Malvaceae and Rutaceae which together constituted 51 per cent of the identified pollen. *A. indica, Cassia auriculata* L., *C. pentrandra, D. regia, Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg., *Jatropha curcas* L., *Malvaviscus arboreus* Cav., *M. paradisiaca, P. ferrugineum, P. juliflora* were recorded as predominant pollen types.

Melissopalynological analysis of 21 honey samples collected from 10 localities of Himalayan region during summer and winter season revealed that dominant species included *Brassica* sp., *Wendlandia* sp., *Solanum* sp., *Ageratum* sp., *Clematis* sp., *Adhatoda* sp., *Mussaenda* sp., *Helianthus* sp., and Papilionaceae, Rubiaceae, Rutaceae and Zingiberaceae members. Analysis also revealed that *Solanum* sp., *Helianthus* sp., *Ageratum* sp., *Caesalpinia* sp., *Polygonum* sp. and *Ocimum* sp. were present as minor pollen types (Singh *et al*., 1994).

2.4 METABARCODING OF POLLEN GRAINS

Genetic barcoding enables researchers to proficiently create taxonomic profiles from multiple pollen samples without specialized palynological training. DNA metabarcoding is the combination of principles of DNA barcoding and next generation sequencing technology which creates opportunity to produce large data on biodiversity (Coissac *et al*., 2012). The pollen DNA barcoding studies previously utilized the nuclear ribosomal DNA marker ITS2 but more recently, it has been demonstrated that plastid DNA (*pt*DNA) can be amplified from pollen, and standard DNA barcoding markers can be effectively used for pollen DNA barcoding and metabarcoding (Hawkins *et al*. 2015, Richardson *et al*. 2015). DNA metabarcoding utilizes a High Throughput Sequencing (HTS) strategies to investigate DNA barcode of all species in a mixed sample of species (Bell *et al*., 2019).

DNA-based identification has the possibility to lessen processing time and elevate the level of species discrimination. Either plastid markers such as *rbcL* (core DNA barcode), *trnH-psbA* and *trnL* (additional markers) or the nuclear ITS and ITS2 region are utilized. ITS2 region can recognize the pollen signature of honey since it can distinguish most plant species (Yao *et al*., 2010).

Six *Apis mellifera* and one stingless honey samples were metabarcoded and the meliponine honey contained 63 distinguishable botanical sources. Pollen analysis yielded four botanical sources *Avena* sp., *Glycine max* (L.) Merr., *Pueraria montana* (Lour.) Merr. and *Elymus* sp. while liquid analysis recognized 53 sources

(Prosser and Hebert, 2017). An evaluation of field observed and metabarcoded sources of bees was done in which results indicated that wildflowers like *Chrysanthemum* sp., *Papaver rhoeas* L. and *Phacelia tanacetifolia* Benth. dominated and unmistakably, more species were identified in metabarcoding (Potter *et al*., 2019).

Twenty five species from 21 genera in 19 families were identified by Gous (2017) with the ITS2 barcoding of pollen samples from *Megachile venusta*. Five species were dominant in the results, namely *Pteris vittata* L. (34.6 per cent), *H. annuus* (32.4 per cent), *Astragalus membranaceus* Moench (17.2 per cent), *Magnolia kwangtungensis* Merr. (3.3 per cent), and *Macrothamnium leptohymenioides* Nog. (3.2 per cent).

Hawkins *et al*. (2015) conducted analysis of nine honey sample by the methods of melissopalynology and metabarcoding. The outcomes demonstrated that a total of 46 plant families from 25 orders were recorded from honeys H1 to H9 using DNA metabarcoding and melissopalynology. DNA metabarcoding identified a greater number of the taxa to species level (31 per cent compared to 27 per cent).

A higher number of taxa at all taxonomic levels were allocated using the DNA sequencing technique compared to light microscopy. More than twice as many families were relegated using sequencing. Only one plant species, *Melilotus officinalis* (L.) Pall., was detected with the light microscopy technique, though 69 plant species were detected with DNA sequencing (Smart *et al*., 2017). Kamo *et al*., (2018) developed metabarcoding techniques in which a combination of *trnL-trnF* and ITS2 proved to be practical for recognizing the honeybee pollen pellets gathered and discovered 25 of 31 taxa (80.6 per cent) could be distinguished using *trnL-trnF* while 24 (77.4 per cent) could be identified using ITS2, and was also able to identify 29 taxa (93.5 per cent) using the combination of these two DNA barcoding locales.

A unique methodology to authenticate honey sources by combination of efficient pollen DNA isolation protocol and of two barcode (*rbcL* and *matK* gene) combinations in identifying the botanical origin of pollen was developed by Manivanan *et al*. (2018). They reported modified CTAB DNA extraction technique combined with two barcode combination to be more precise.

In spite of the potential advantages of DNA metabarcoding over visual examination of pollen, the method has not been thoroughly assessed in terms of how well it works qualitatively or quantitatively. Automated systems can gather and store images of all grains located and classified and this offers the potential of relating later on, in view of restructured reference data, including the potential for better discrimination, bringing older sites up to the similar identification standard as present sites (Holt and Bennett, 2014). With a better understanding of partialities related to DNA isolation, preservation and PCR amplification, it might be possible to correct for these biases, and precisely determine the relative proportions of species within a mixture (Bell *et al*., 2016).

2.5 FORAGING ACTIVITY OF STINGLESS BEES

According to Biesmeijer and Toth (1998), bees collecting pollen were typically active for 1 to 3 hours a day in the early morning. They also reported that nectar foragers collected nectar actively for 4 to 10 hours per day. They started collecting every morning between 0530 and 0730 h and stopped between 1230 and 1620 h. Pollen-nectar foragers collected pollen first from 0530 h up to 0900 h and then changed to nectar collecting lasting until 1700 or 1800 h.

Gilbert (1973) studied the foraging activity of *Trigona fulviventris* Guerin-Meneville during the dry season. The highest activity period of *T. fulviventris* was in the morning hours starting at about 0530 h, reaching a peak of activity about 0600 h (80 bees/min) continuing until about 1000 h (20 bees/min) followed by a lower activity level until after 1500 h, and then a second peak at about 1700 h (40 bees/min). Thereafter, departure rate declined rapidly and arrival rate inclined by 1750 h and later, all activity had stopped.

The foraging activity study of *Melipona asilvai* Moure was carried out in a sub humid area of Atlantic Rain Forest during the rainy season (April to August) and dry season (September to March). The nest activity during the dry season initiated around 0530 h and the peak activity of outgoing foragers occurred between 0700 and 0800 h while in the rainy season, the outgoing activity started between 0600 and 0900 h but did not produce a clear peak of activity. In both seasons, foraging trips ended around 1800 h. Liquid foragers were more active during the dry season with peak activity at 0700 h whereas no significant peak in activity was observed during the rainy season. Pollen collection was more during the dry season compared to the rainy season and the peak occurred during the first hours of the morning and decreasing by the afternoon (do Nascimento and Nascimento, 2012).

de Bruijn and Sommeijer (1997) studied four species of stingless bees both in natural and greenhouse conditions. Pollen collection started earlier in the day than nectar. *M. fasciata* and *M. beecheii* collected pollen usually before sunrise and most frequently in the very early morning hours. In *T. angustula* pollen foraging started at 0700 h and the peak was around 1100 h and gradually decreased until sunset. Nectar foraging in *T. angustula* started at 0800 h increasing gradually until 1300 h and decreasing until 1800 h. In greenhouse, only pollen foraging differed from natural conditions among the species.

According to Ferreira Junior *et al*., (2010) in southern Brazil, the pollen was preferentially collected by the bees of *Melipona bicolor schencki* Gribodo in the morning during all four seasons, continuing till the early afternoon in summer and winter. Returning flights with nectar/water increased in frequency during the day in all the seasons and were intense during all flight hours in spring and summer.

Roubik *et al*. (1986) in a comparative study on foraging activity of stingless bee and *Apis mellifera* Linnaeus reported that stingless bees appeared to compensate for competition by altering their foraging performance and peaks of intense. Foraging of stingless bees was rare and there was a conspicuous lack of such peaks when honey bees were also foraging.

In Brazil, a comparative study of *Plebeia remota* Holmberg foraging activity during both diapause and reproductive phase was conducted. The total number of bees collecting resources in the reproductive phase increased until 0900 h with peak activity between 0900 and 1100 h, decreased until 1300 h then remaining a constant for the rest of the day. The total number of foragers during the diapause increased from 0800 to 1100 h, remained constant from 1100 to 1300 h and then decreased till 1800 h. The nectar collection increased from 0800 to 1100 h then remained constant until 1600 h during reproductive phase and in the diapause, it increased from 0800 to 1100 h and showed a peak between 1100 and 1200 h. Whereas the pollen collection showed a peak at the beginning of the morning, between 0800 and 1000 h in the reproductive phase while in diapause peak was between 0800 and 1100 (Nunes-Silva *et al*., 2010).

Eltz et al. (2002) reported that the tube traffic in colonies of *T. collina* was more during 0700 h to 1000 h in the morning. The pollen collection was more active in the early morning hours, although from 1000 h to 1800 h the activity did not vary between sites.

Foraging of three colonies of *Heterotrigona itama* Cockerell was recorded from 0800 to 1700 h in Terengganu, Malaysia during 2016. The outgoing forager activity commenced at 0800 h where most foragers returned to the nest carrying nectar throughout the early morning, between 0800 and 0900 h. The activity increased at 1000 h for both outgoing and incoming and the most active foraging period was late morning. The pollen collection by the forager was highest between 0900 h and 1100 h while the nectar was collected by the forager throughout the day. The higher mean number of outgoing forager and returning forager was in May and lowest in April. The mean number of outgoing and returning foraging behaviour was higher in forest area than agriculture area (Jaapar *et al*., 2018).

Nagamitsu and Inoue (2002) conducted a study in which foraging activity of selected colonies of *T. collina*, *T. melanocephala*, *Trigona rufibasalis* Cockerell, *Trigona thoracica* Smith and *T. melina* was observed in three distinct periods each

in 1994 (June, August and September), and in 1996 (May, June and August) and at 0730 h, 1030 h, and 1430 h on a single day. The highest frequency of forager returns was at 1030 hr and was lowest at 1430 h. At 0730 h, pollen foragers were more than nectar foragers. At 1430 h, nectar foragers were most, and pollen foragers were fewest.

Saufi and Thevan (2015) demonstrated that the most astounding frequencies for all foraging activities of *Geniotrigona thoracica* Smith was in the dry season. The outgoing foragers and incoming foragers with pollen were highest in the time of 1000 h until 1200 h. The foragers with debris were highest in the early morning, 0800 h until 1000 h and incoming foragers were maximum at 1200 h until 1400 h. The lowest rate of foraging was from 1600 h until 1800 h.

Bartareau (1996) conducted studies on *Trigona (Tetragonula) carbonaria* Smith in the grassland of Queensland, Australia. The number of bees per feeder steadily increased during the day to reach a maximum between 1500 and 1600 h and rapidly decreased from 1700 to 1800 h.

In a study conducted in Terengganu, three colonies of *H. itama* were observed in all months from early morning to the mid-day from April to June 2013. The highest peak time of outgoing foragers was at 1000 h during May and June, while in April, it was at 1200 h. The lowest number of outgoing foragers was observed in the late afternoon in all months. In April and June, the peak time of incoming foragers with pollen loads was 0900 h while in May it was 1100 h. The number of total incoming foragers was lower in the early morning at 0800 h, but suddenly inclined to the highest at 0900 h and slowly declined from 1100 to 1800 h to the lowest number. The peak time of nectar foraging was at 1300 h in April, while in May and June it was at 0900 h (Ghazi *et al*., 2014).

Four colonies of *Trigona* (*Tetragonula*) *minangkabau* Sakagami and Inoue one each of *Trigona* (*Heterotrigona*) *itama* and *Trigona* (*Trigonella*) *moorei* Schwarz was observed in Sumatra. Average total flights per day per colony was

1200 in *T. minangkabau*, 2400 in *T. moorei* and 7000 in *T. itama*. Nectar collection in *T. itama* was most during midday and of *T. minangkabau* and *T. moorei* were uniform during the daytime. Pollen collection was more active in the early morning by the three species (Inoue *et al.*, 1985).

Singh and Khan (2015) reported that the incoming bees with pollen were maximum and identical at 0800 h and 0400 h and minimum at 1200 h. The number of incoming bees with nectar was highest at 0400 h followed by 1200 h and 0800 h.

Saravanan and Alagar (2007) reported that *T. iridipennis* performed 3048 to 11028 flights per day and flight activity was maximum in morning hours and evening hours with a reduction from 1300 to 1500 h. Greatest activity for the foraging of pollen and nectar was recorded between 0800 and 1200 h. Pollen foraging, which started around 0700 h, peaked between 0800 to 1100 h and after that gradually declined until dusk. Foraging for nectar increased gradually after 0800 h without showing a distinct peak. Workers of both the colonies made 60 to 70 per cent of their trips for nectar foraging and 20 to 25 per cent trip for pollen foraging, wherein resin accumulation accounted only for just 3 to 10 per cent of their absolute number of flights.

Mohapatra *et al*. (2014) as observed in Bhubaneswar bees of *T. iridipennis* recorded highest activity in June during which 9.9 nectar gatherers and 6.4 pollen gatherers returned to the hive every minute. The foraging movement was low during the period of August (6.4 nectar gatherer and 3.3 pollen gatherer). Peak foraging activity was recorded at 1100 h of the day when 12.1 nectar gatherer and 7.9 pollen gatherer per minute came back to the hive. Foraging activity was least at 0700 h when 5.9 nectar gatherer and 3.1 pollen gatherer was recorded.

According to Devanesan *et al*. (2002), the foraging activity of *T. iridipennis* started around 0700 hr after which a gradual rise was observed reaching the first peak at 1200 hr. A decline in activity was then observed followed by an increase in activity which reached a second peak at 1500 hr. The pollen foraging was more

observed during the morning and the nectar foraging during noon. The mean of incoming bees with pollen load was 17.9 during the first peak compared with 13.9 during the second peak. The mean of incoming nectar collectors were 11.5 and 10.8 during the first and second peak respectively.

Foraging activity of stingless bee, *T. iridipennis* was studied at Dharwad during 2006-07 and outgoing bees and incoming bees with pollen load (pollen foragers) and without pollen load in three seasons were recorded. In every season the lowest activity of outgoing was recorded at early morning 0600 h. The highest peak of activity was at 1200 h and 1000 h during monsoon and summer season respectively. During winter the highest peak was during 1200 h and 1400 h. During monsoon, the pollen foraging increased from morning reaching the higher levels at 1000 h, 1200 h and 1400 h. During winter the activity reached the highest peak at 1200 h whereas during summer the activity attained the highest peak at 1000 h. In every season the lowest number of bees without pollen load was observed in early morning hours and declined towards the end of the day. The activity increased and reached the highest peak at 1200 hr during monsoon and winter. During summer comparatively higher activity was recorded at 1000 and 1200 hr (Danaraddi *et al.*, 2011).

Vazhacharickal and Jose (2016) measured bee foraging activity in four colonies of *T. iridipennis* from 0600 to 1910 h. In all the colonies, bee activity started between 0600 to 0700 h and ended by 1900 to 1910 h. Two peaks were obtained, one from 1000 to 1010 h and the other 1400 to 1500 h although, in one colony, the morning peak was from 1100 to 1110 h and the afternoon peak was 1500 to 1510 h.

Studies on foraging pattern of *Trigona laeviceps* Smith revealed that peak foraging activity of incoming and outgoing foragers was at 1100 h, then decreased. Maximum activity of pollen foragers were observed during morning around 1000 h and nectar foragers at mid-day 1300 h. Peak activity was observed in month of April and least in month of January (Managanvi *et al*., 2012).

Materials and Methods

3. MATERIALS AND METHODS

The present study was carried out under the title "Melissopalyological studies on stingless bees *Tetragonula travancorica* (Apidae; Meliponinae)" at AICRP on Honey bees and Pollinators, Department of Agricultural Entomology, College of Agriculture, Vellayani and in selected colonies of stingless bees from Thiruvananthapuram district during 2017-20.

3.1 SELECTION OF APIARIES

The apiaries were selected from different locations of Thiruvananthapuram district based on elevation of the region (Fig.1). Thiruvananthapuram is the southernmost district of Kerala lying between North Latitude 8^0 17'and 8^0 51' and East Longitude 76^0 41' and 77^0 17', which has a huge coastal margin and can be divided geographically into three sub-micro regions, *viz.* lowland, midland and upland regions. The regions with an elevation below 20 m above mean sea level are lowlands, 20-100 m above mean sea level are midlands and more than 100 m above mean sea level are uplands. The regions coming under midlands and highlands were selected, as most of the stingless beekeeping is practiced over these topographic areas. Among the total of eighteen locations, fourteen were selected from midland region and four were highlands. The details of the locations of the apiaries selected are given in Table 1.

3.2 METHOD OF SAMPLE COLLECTION

Samples of honey and pollen loads were collected from the selected locations (Plate 1) in three seasons namely, Northeast monsoon season (October-December), Dry season (January- May) and Southwest monsoon season (June-September). Both honey and pollen samples were taken from randomly selected colony in every season.

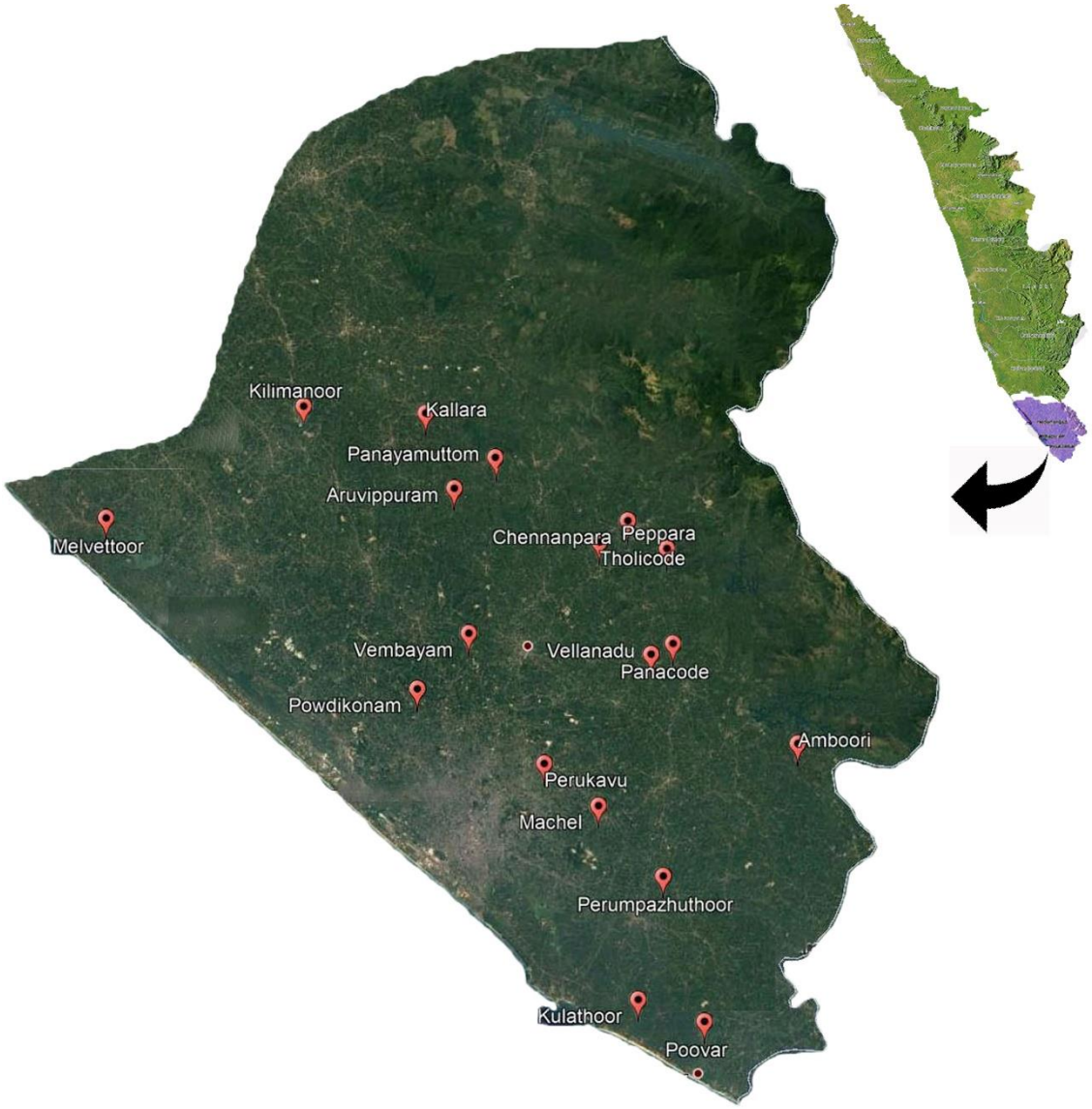


Fig 1. Localities of sample collection in Thiruvananthapuram district

Table 1. Details of selected locations in Thiruvananthapuram district for collection of honey and pollen samples of *Tetragonula travancorica*.

Sl. No.	Location	North Latitude	East Longitude	Altitude (in metres)	Category (Mid/High)
1.	Kilimanoor	76°52’48’’	8°46’1’’	75	Mid
2.	Panacode	77º5’45’’	8º34’43’’	72	Mid
3.	Kaviyode	77º4’45’’	8º34’43’’	67	Mid
4.	Amboori	77°9’34’’	8°30’11’’	60	Mid
5.	Perumpazhuthoor	77º4’7’’	8º25’53’’	32	Mid
6.	Kulathoor	77º 4’ 48”	8º19’35”	37	Mid
7.	Poovar	77º4’40’’	8º20’4’’	31	Mid
8.	Machel	77°1’61”	8°29’2”	29	Mid
9.	Thekkada	76°57’52’’	8°37’29’’	81	Mid
10.	Powdikonam	76°55’38”	8°34’38”	63	Mid
11.	Panayamuttom	77º 0’ 5”	8º42’46”	69	Mid
12.	Aruvippuram	76°58’15’’	8°42’5’’	51	Mid
13.	Melvettoor	76º44’31’’	8º43’11’’	56	Mid
14.	Perukavu	77º0’4’’	8º30’41’’	51	Mid
15.	Peppara	77º6’4’’	8º38’29’’	133	High
16.	Kallara	76º57’58’’	8º45’30’’	146	High
17.	Tholicode	77º 3’ 31”	8º 39’ 9”	113	High
18.	Chennanpara	77º4’48’’	8º39’37’’	124	High

Plate 1. Visited apiaries and hives of farmers

3.2.1 Collection of pollen loads

Pollen was collected from the pollen storage pots (Plate 2.) inside the hives. Composite sampling was done from three different regions inside the hives. Pollen was collected from pots into 50 mL plastic containers which were labelled and stored (Plate 3a.) in refrigerator for further studies.

3.2.2 Collection of honey samples

Honey storage pots were collected from hives in 50 mL plastic containers and honey was collected later with pasteur pipettes into 15 mL glass vials (Plate 3b.). These were labelled and stored for further analysis.

3.3 LABORATORY ANALYSIS OF SAMPLES

The collected samples of honey and pollen were subjected to procedure of acetolysis (Plate 4 a-h) in the laboratory before the slide preparation for pollen analysis. Acetolysis is carried out in-order to remove molecules especially carbohydrates on the pollen grain and which makes the descriptive characters clear on the surface (Erdtman, 1960; Jones, 2014). The chemicals used for the procedure included Acetic anhydride, conc. Sulphuric acid and Glacial acetic acid and instruments involved were Eppendorf microcentrifuge and heat block.

3.3.1 Procedure for analysis of honey

The procedure followed for analysis of honey is following:

2 mL honey + 8 mL distilled water

↓ Centrifuged @ 5000 rpm (5 min)

Supernatant is decanted and mixed with distilled water

↓ Centrifuged @ 5000 rpm (5 min)

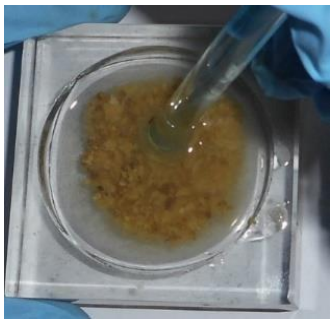
Plate 2. Method of sample collection

a.

b.

Plate 3 a. Collected and labelled samples of pollen b. Collected and labelled samples of honey

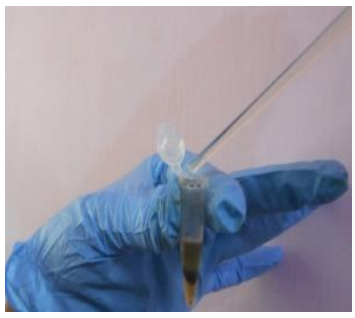
a. Pollen first crushed in alcohol, centrifuged and washed



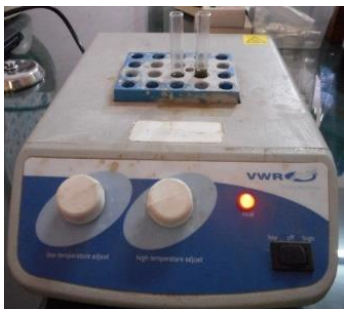
b. Then crushed in glacial acetic acid

c. The mixture transferred to microcentrifuge tubes, centrifuged and sediment obtained

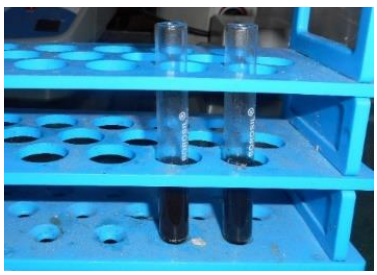
d. Acetolysis mixture freshly prepared (Acetic anhride : Conc. Sulphuric acid, 9:1)



e. Acetolysis mixture added to sediment



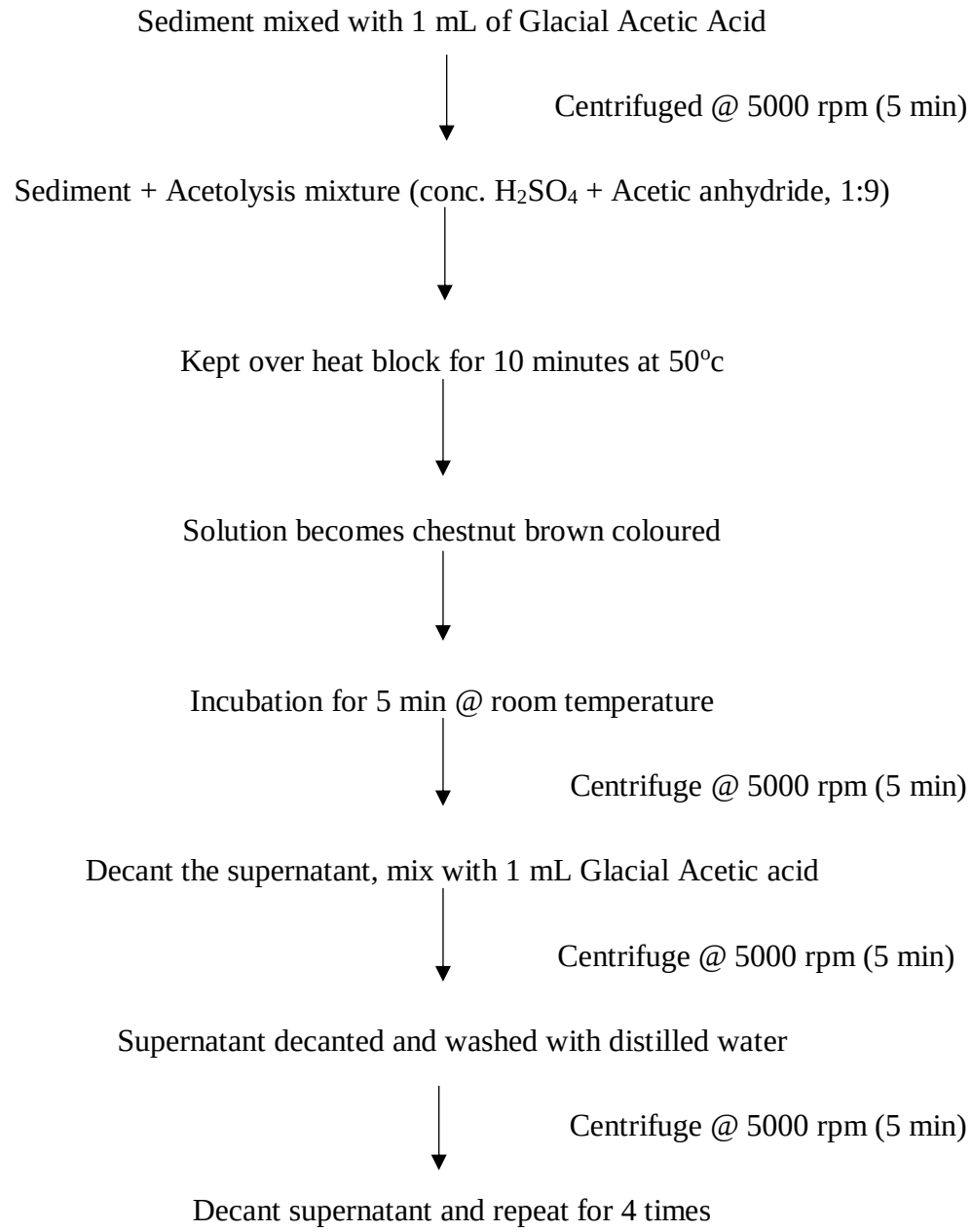
f. Heated for 10 min at 45 ^{o}C with intermittent stirring



g. Incubated for 5 minutes, centrifuged and sediment taken

h. Glacial acetic acid added, centrifuged and sediment washed 5 times

Plate 4. Methodology for acetolysis procedure



The sample prepared is mixed with glycerine and mounted with glycerine jelly for slide preparation after decantation of glycerine.

3.3.2 Procedure for analysis of pollen load

The pollen samples collected from pots using clean forceps, was crushed in alcohol and centrifuged for 5 min @ 5000 rpm and washed. The sediment obtained

after decanting the supernatant is subjected to acetolysis according to the procedure in 3.3.1

3.3.3 Preparation of permanent slides

Permanent slides from the acetolysed samples were mounted using glycerine jelly which was prepared. The method of preparation and the reagent required are as follows:

3.3.3.1 Reagents

Glycerin	-	35 mL
Gelatin	-	10 g
Water	-	30 mL
Phenol crystals	-	3 g

3.3.3.2 Method of preparation

Water was boiled at 100^{o}c and gelatin was mixed while stirring. All the remaining components were mixed while it was still hot. It was then kept for cooling and solidification. Phenol is a preservative and prevents the fungal growth in the medium. Slides were prepared by taking pollen over the small bits of glycerine jelly and placing on it. The dimension of slides used were 75 x 25 mm size and 1-1.2 mm thickness. The cover slips used were of 0.08mm size. Cover slips were sealed with paraffin wax later.

3.4 IDENTIFICATION AND CHARACTERISATION OF POLLEN

3.4.1 Morphological categorisation

Pollen grains mounted on the slide were identified based on the references available. The characters were defined according to the 'Glossary of pollen and spore terminology' (Punt *et al*., 2007). Morphological characters involved in the description of different pollen types are given in detail in Table 2.

Table 2. Characteristic depiction of pollen grains

Characteristics	Types	Description
Shape	Oblate	P/ E 0.5-0.75
	Prolate	P/ E 1.33- 2
	Spheroidal	Circular or P/E equal to one
	Peroblate	P/E less than 0.5
	Perprolate	P/E more than 2
Size and symmetry	Radial	Two or more plane of symmetry
	Bilateral	Single plane of symmetry
Pollen association	Monads	Pollen as an individual unit
	Dyads	Two spores united as a dispersal unit
	Tetrads	Four spores united
	Tetrahedral	Multiplanar tetrad in which each spore is in contact with other three
	Tetragonal	Uniplanar tetrad in which four members in contact at centre of tetrad
	Rhomboid	Uniplanar tetrad in which proximal faces of two spores are in direct contact and other two separated
	Polyads	More than four pollen grains in dispersal unit
Aperture type	Colpi	Elongated aperture with l/b ratio greater than 2
	Pore	Circular or elliptic with l/b ratio less than 2
	Sulcus	Elongated latitudinal ectoaperture at distal or proximal pole
	Ulcus	Rounded ectoaperture at proximal or distal pole
Aperture position	Proximal	Surface that face towards centre of tetrad during development
	Distal	Surface that face away from centre of tetrad during development

	Zonal Panto	Located equatorially Aperture spread over the surface
Aperture number	Mono, Di, Tri, Tetra, Penta, Hexa etc.	The number of aperture on the pollen
Exine ornamentation	Foveolate Fossulate Rugulate Reticulate Striate Retipilate Verrucate Clavate Areolate Gemmate Baculate Psilate	More or less round depressions with lumina more than 1 µm diameter Elongated irregular groove Irregular pattern intermediate between reticulate and striate Network like pattern with lumina wider than 1 µm Parallel elements separated by grooves on exine Reticulum formed by two rows of pila Wart like element more than 1 µm wide broader than high Club shaped element higher than 1 µm, thicker at apex than base Circular or polygonal areas separated by grooves forming negative reticulum Elements higher than 1 µm, same width as height Cylindrical element more than 1 µm and less in diameter Smooth surface

3.4.2 Microphotography and measurements

The photographs of pollen grains were taken under the compound microscope Leitz Orthoplan. The images were obtained under a magnification of 800X and were captured using the software LAS V3.6 using the connected Leica DFC295 camera. The images were taken in different focus plains and were focus stacked by using Helicon focus stacker, into a composite image. Measurements were manually taken by using ocular micrometer.

3.5 POLLEN CLASS AND FREQUENCY DISTRIBUTION

Three slides were prepared from samples of each location and all the three were observed for recording pollen count. From each slide, five fields were selected and pollen density was estimated by converting it to the whole slide area.

The pollen percentages were calculated from the count of 1200 or more pollen grain and the percentage of a particular pollen grain type was obtained by the following formula:

$$\text{Percentage of a pollen type} = \frac{\text{Number of pollen grains of that pollen type X 100}}{\text{Total number of pollen grains}}$$

3.5.1. Pollen class types

Pollen class frequencies were categorized from the recorded pollen count in samples and the following representative terms were used;

Predominant pollen	-more than 45 per cent of the pollen grains counted
Secondary pollen	- 16 to 45 per cent of the pollen grains counted
Important minor pollen	- 3 to 15 per cent of the pollen grains counted
Minor pollen	- less than 3 per cent of the pollen grains counted

From these classes, the most significant as well as minor source to honey bees was identified and pollen spectrum was constructed for seasons.

3.5.2. Pollen grain frequencies

The pollen grain frequencies were determined from the pollen count according to Louveaux *et al*., 1978 and following are the terms used to describe frequencies;

Very frequent	-	more than 45 per cent of the total
Frequent	-	16 to 45 per cent of the total
Rare	-	3 to 15 per cent of the total
Sporadic	-	less than 3 per cent

3.5.3. Seasonal variation

The occurrence of different pollen types of all locations together in each of the season, North-east monsoon, Dry and South-west monsoon, was recorded and enlisted separately to observe the seasonal variation.

3.6. METABARCODING

The pollen samples obtained from the locations of collection during all three seasons were made into a composite sample of each season. This sample was subjected to metabarcoding, protocol of which is elaborated in the following steps.

3.6.1. Genomic DNA isolation

Genomic DNA was isolated from the pollen samples by using Nucleospin DNA extraction kit. DNA extraction was carried out using 200 mg dry weight of pollen sample.

3.6.1.1 Homogenizing sample

The sample upto 200 mg dry weight (lyophilised) was homogenised with appropriate homogenising methods.

3.6.1.2 Cell lysis with Buffer PL1

The resulting powder from above step was transferred into a new tube and 400 µL Buffer PL1 and 10 µL DTT (1 M) was added. This was followed by 10 µL RNase A solution and sample was mixed thoroughly. The suspension was incubated for 1 hour at 65 ^{o}C

3.6.1.3 Filtration/Clarification of crude lysate

A Nucleospin filter was placed into a new collection tube (2 mL) and lysate was loaded into the column and centrifuged for 2 min at 11,000 x g. The clear flow-through was collected and filter was discarded.

3.6.1.4 Adjusting DNA binding conditions

The aliquot obtained was added with 450 µL Buffer PC and was mixed thoroughly by vortexing.

3.6.1.5 Binding DNA

A Nucleopsin Plant II column was placed into a new collection tube of 2 mL and 700 µL of sample was loaded. This was centrifuged for 1 min at 11,000 x g and flow through was discarded.

3.6.1.6 Washing and drying silica membrane

Initially, 400 µL Buffer PW1 was added to the Nucleopsin Plant II column and centrifuged for 1 min at 11,000 x g and flow through was discarded. In second wash 700 µL Buffer PW2 was added to the Nucleopsin Plant II column and centrifuged for 1 min at 11,000 x g and flow through was discarded. During third wash another 30 µL Buffer PW2 was added to the Nucleopsin Plant II column and centrifuged for 2 min at 11,000 x g to remove the wash buffer and to dry out the silica membrane completely.

3.6.2. PCR

The DNA sample obtained by isolation had a concentration of 1 ng/ µL. DNA barcoding analysis was carried out using regions of Ribulose bisphosphate carboxylase large chain a and b (rbcLa, rbcLb) in cpDNA, coding locus psbA gene in the photosystem II, Internal Transcribed Spacer 4 and 5 region of nuclear ribosomal DNA (ITS4, ITS5), protein coding Maturase K3 and 4 (matK) region of plant plastid and trnH intron region of chloroplast. Five primer combinations rbcLa forward and reverse (743 bp), rbcLb forward and reverse (861 bp), psbA-trnH (288-802 bp) ITS4- ITS5 (554-1103 bp) and matK3- matK4 (886 bp) were used for PCR amplification and sequencing.

rbcLa (forward): 5' ATG TCA CCA CAA ACA GAG ACT AAA GC 3'

(reverse): 3' TCG CAT GTA CCT GCA GTA GC 5'

rbcLb (forward): 5' AGA CCT WTT TGA AGA AGG TTC WGT 3'

(reverse): 3' TCG GTY AGA GCR GGC ATR TGC CA 5'

ITS 4 (forward): 5' TCC TCC GCT TAT TGA TAT GC 3'

ITS 5 (reverse): 3' GGA AGT AAA AGT CGT AAC AAG G 5'

matK3 (forward): 5' CGT ACA GTA CTT TTG TGT TTA CGA G 3'

matK4 (reverse): 3' ACC CAG TCC ATC TGG AAA TCT TGG TTC 5'

trnH (forward): 5' CGC GCA TGG TGG ATT CAC AAT CC 3'

psbA (reverse): 3' GTT ATG CAT GAA CGT AAT GCT C 5'

The amplification reaction contained 25 µL of 2x PCR mix (pre-mixed 2x concentration of Taq DNA polymerase (0.05 U/ µL), $MgCl_2$ and dNTP), 2 µL each of forward and reverse primer, 4 µL of DNA template, 21 µL of nuclease free water which made up a total of 50 µL. PCR cycles consisted of an initial denaturation

step at 94 °C for 4 min, 35 cycles of denaturation (30 sec at 94 °C), annealing (30 sec at 50 °C), extension (1 min 30 sec at 68 °C) and a final extension (5 min at 68 °C) and then hold at 4 °C till used for gel electrophoresis

The PCR products were checked by electrophoresis on 2 per cent (w/v) agarose gel and three primer combinations were found to be having amplicons (Plate 5).

3.6.3. End repair/dA tailing

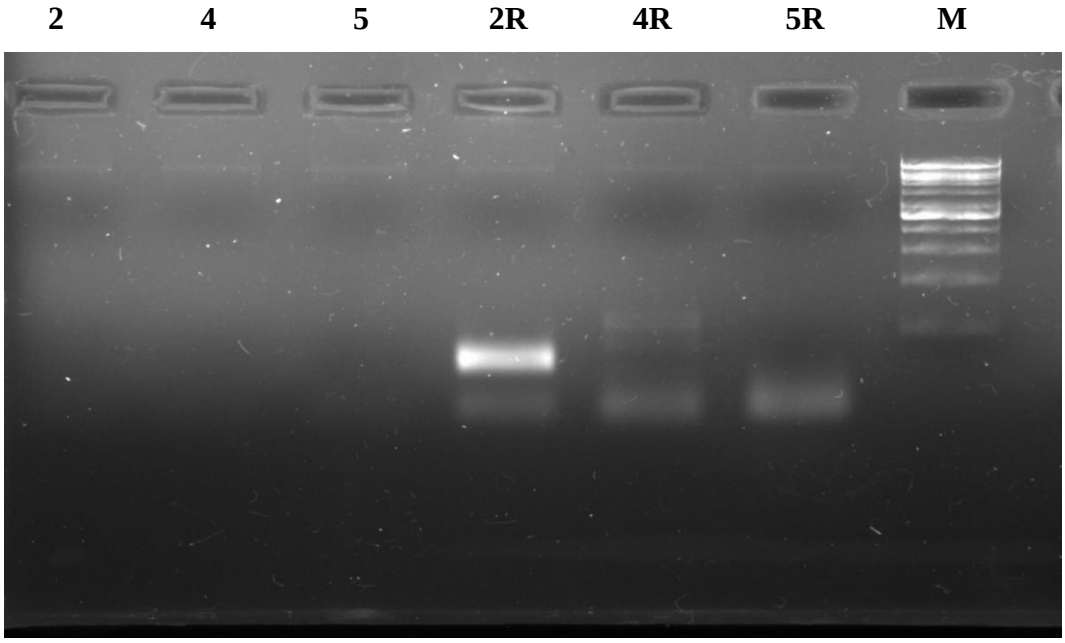
PCR product from all three PCR primer pairs (Plate 5) were pooled together and pooled DNA was subjected to end repair/dA tailing using 3 µL of UltraII End prep enzyme mix in a 60 µL reaction volume in a 0.5 mL PCR tube for 5 min at 20°C and for 5 min at 65°C using thermal cycler. The modified DNA fragments were purified using 60 µL AMPure XP beads, incubated, repeated the step and the DNA was eluted in a 25 µL volume of MQ water. Recovered DNA was quantified using Qubit reader (ThermoFischer Scientific).

3.6.4. Barcode labelling

Five hundred ng of end prepped DNA was coupled with a barcode where each barcode represented one sample. Barcode was ligated using 2X ligation MasterMix supplied by NEB. Ligation reaction was carried out at room temperature using 2.5 µL Native Barcode and 25 µL Blunt/TA Ligase MasterMix while incubated for 10 min in a 50 µL reaction volume. The barcode ligated fragments were purified using 50 µL AMPure XP beads, incubated, repeated the step and the DNA was eluted in a 26 µL volume of MQ water. Recovered DNA was quantified using Qubit reader (ThermoFischer Scientific).

3.6.5. Barcode Adapter ligation

To the barcode labelled DNA, barcode adapter was ligated using 2X ligation MasterMix supplied by NEB. The ligation reaction was carried out at room temperature while incubated for 10 min using 750 ng of pooled samples (750ng/24)



2 –psbA & trnH

4 – ITS5 & 4

5 – matK3 and K1

2R- Reamplification of 2

4R - Reamplification of 4

5R - Reamplification of 5

M- 1 Kb molecular marker

Plate 5. PCR amplification of pollen DNA using metabarcode primers

in 50 µL, 20 µL of barcode adapter mix (BAM), 20 µL NEBNext Quick Ligation Reaction Buffer (5X) and 10 µL Quick T4 DNA Ligase. After the ligation reaction the DNA fragments were purified using 40 µL AMPure XP beads incubated on rotator mixer and 140 µL of ABB buffer and the DNA was eluted in a 15 µL volume of elution buffer. After incubation for 10 min one µL of recovered ligated DNA was quantified using Qubit reader (ThermoFischer Scientific).

3.6.6. Priming and Loading the SpotON flow cell

The Sequencer MinION was connected to SpotON flow cell and primed using 1mL of Primer buffer for 5 minutes at RT and into the primed flow cell 75 µL of pooled library (430ng) prepped was added through the SpotON sample port in a dropwise manner. The SpotON sample port was replaced, priming port closed and MinION lid was replaced.

3.6.7. Sequencing run and basecalling

After connecting the flow cell to the computer, the MinKNOW programme was started. The sequencing was done in the FASTQ barcoding mode for 48 hrs and the basecalling was done using the software GUPPY.

3.6.8. Data analysis

After basecalling the DNA sequence data files obtained as FASTQ format were uploaded to NCBI website for BLAST analysis.

3.7 ABSOLUTE POLLEN DENSITY OF HONEY

The total number of pollen grains in honey samples obtained in different seasons were observed and recorded. The pollen from 1 mL of honey was separated by centrifugation @ 5000 rpm and suspended in 1 mL distilled water. Later the total number of pollen grains were counted in Haemocytometer by pipetting out 100 µL from the sample. The average of one column was taken and total pollen density was calculated by the formula:

$$\text{Total pollen count (in 1 mL of honey)} = \frac{\text{Total count in one column of haemocytometer X 1000}}{0.1}$$

The honey samples were then categorised into groups (Maurizio, 1976; Louveaux *et al*., 1978) based on the absolute pollen count as follows:

Group I	-	< 20,000
Group II	-	20,000- 1, 00,000
Group III	-	1, 00,000- 5, 00,000
Group IV	-	5, 00,000- 10, 00,000
Group V	-	> 10, 00,000

3.8. FORAGING ACTIVITY

The foraging activity was studied at the College of Agriculture, Vellayani, Thiruvananthapuram District, from November 2018 to August 2019. Two of the wooden hives installed inside the campus were selected for the study (Plate 6) and outgoing and incoming foraging bees were counted during every hour for 5 minutes from morning 0600 h to evening 1800 h. The study site is surrounded by Vellayani lake on three sides and has an area of 2.52 square km. The campus is located in a tropical monsoon region with seasons being divided into three major parts, namely Northeast monsoon (November to December), Dry season (January to May) and Southwest monsoon (June to August). The majority of the rain in this area is influenced by southwest monsoon winds and lesser portion of monsoon is due to northeast winds.

The outgoing foragers, incoming foragers with pollen, foragers without pollen, incoming resin or mud foragers, guard bees flying and landing at the entrance and outgoing foragers carrying waste material out of the hive were recorded separately. To calculate the actual incoming foragers without pollen, the

Plate 6. Hives observed for foraging activity

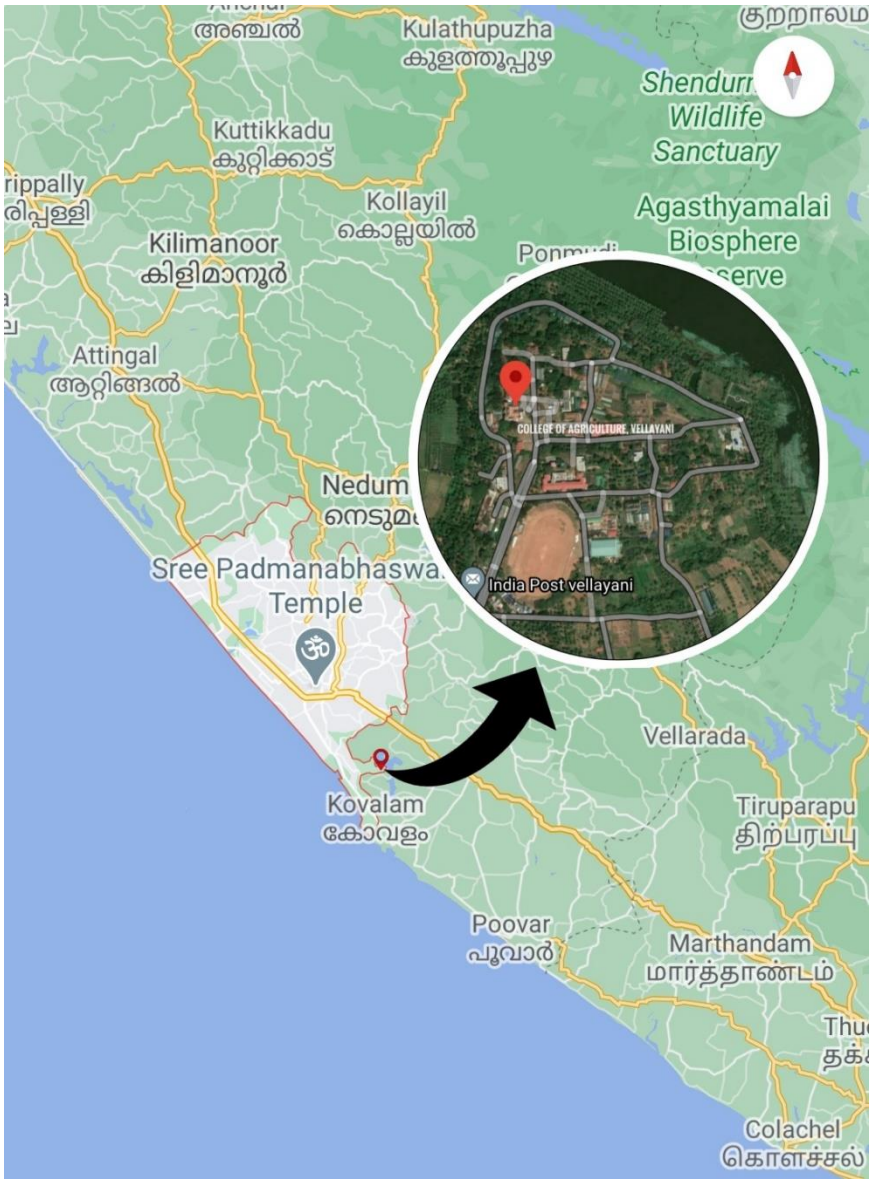


Fig 2. Location of foraging activity studies

number of bees removing garbage and the landing guard bees were subtracted from total count of incoming bees without pollen/resin in every hour.

The actual number of incoming bees without pollen

= total number of incoming bees without pollen/resin- (number of garbage remover bees + number of guard bees landing back).

3.8.1 Statistical analysis

The count values obtained in every season were subjected to two way factorial analysis considering months (M) and time (T) as the two factors. The analysis was conducted after carrying out square root transformation on the mean values obtained from two hives. The analysis was carried out using statistical package in SAS software.

Results

4. RESULTS

The results of the study "Melissopalynological studies on stingless bee *Tetragonula travancorica* (Apidae; Meliponini)" is presented below under the following headings of this chapter.

4.1 POLLEN IDENTIFICATION AND CHARACTERIZATION

The pollen and honey analysis made clear the presence of 115 pollen types over the three seasons, namely, northeast monsoon season (October – December), dry season (January – May) and southwest monsoon season (June – September). The pollen types thus obtained were recognized to the species, generic or family level. The photographic images of the pollen types during analysis are given in Plate 7-18.

The size, shape, ornamentation and number of aperture were considered and pollen were identified upto the possible level of hierarchy. It was then described based on the characters in the 'Glossary of pollen and spore terminology and published papers on the genus' (Punt *et al*., 2007). Identified pollen included sixty seven at species level, sixteen at generic level and ten at family level. Twenty two pollen types remained unidentified. The characteristics of the identified pollen types as observed are given in the Table 3.

4.2 DISTRIBUTION OF POLLEN TYPES OVER SEASONS

The percentage distribution of pollen types in the pollen and honey samples from eighteen locations during all three seasons, northeast monsoon (October – December), dry season (January – May) and southwest monsoon (June – September) was found out to understand the plants contributing greatest to the sources of pollen and honey.

Table 3. Characteristics of pollen types identified

Plate No.	Scientific name	Family-Subfamily	Character
7a.	*Mimosa pudica*	Fabaceae-Mimosoideae	Pollen grains are tetrahedric tetrads, heteropolar, oblate spheroidal, acalymmate and 12 porate, each cell with 3 pores, psilate exine, sexine and nexine indistinct.
7b.	*M. diplotricha*	Fabaceae-Mimosoideae	Pollen grains are tetrads elliptic, circular amb, prolate spheroidal shape, tetrapantoporate, circular pori, psilate exine, sexine and nexine indistinct.
7c.	*Milletia pinnata*	Fabaceae-Faboideae	Pollen grains are monad, bilaterally symmetrical, circular amb, prolate–prolate spheroidal, tricolporate, angulaperturate, ora circular, punctitegillate, exine faintly reticulate.
7d.	*Peltophorum pterocarpum*	Fabaceae-Caesalpinioideae	Pollen grains are monad, isopolar, bilaterally symmetrical, triangular amb with rounded angles, oblate spheroidal shape, trizonocolporate, planaperturate, ora circular, exine reticulate, sexine thicker than nexine.
7e.	*Acacia mangium*	Fabaceae-Mimosoideae	Pollen grains are polyad, 16 celled, circular amb, elliptic shape, diameter 41-42.15 µm, individual grain aperture 4 porate, surface faintly foveolate.
7f.	*A. latronum*	Fabaceae-Mimosoideae	Pollen grains are polyad, 16 celled, circular amb, elliptic shape, diameter 55-57.65 µm, individual grain aperture 4 porate and surface foveolate.
7g.	*A. catechu*	Fabaceae-Mimosoideae	Pollen grains are polyad, 16 celled, circular amb, elliptic shape and diameter 43.1-44.54 µm, individual grain aperture indistinct and surface foveolate.
7h.	*Delonix regia*	Fabaceae-Caesalpinioideae	Pollen grains are monads, isopolar, circular to subtriangular amb, oblate spheroidal shape, tri zonocolporate, planaperturate, ora lalongate, exine retipilate, sexine thicker than nexine

8a.	*Cocos nucifera*	Arecaceae-Arecoideae	Pollen grains are monad, heteropolar, bilaterally symmetrical, amb elliptical, monocolpate, pantocolpate aperture, colpi with round edge, faintly reticulate surface pattern.
8b.	*Borassus flabellifer*	Arecaceae-Coryphoideae	Pollen grains are monads, elliptical amb, monocolpate, colpi tapering towards end, sexine tectate, verrucate sexine, tectum minutely reticulate, muri simplibaculate, sexine thicker than nexine.
8d.	*Elaeis guineensis*	Arecaceae-Arecoideae	Pollen graisn are monads, radially symmetrical, triangular amb with rounded angles, trichotomosulcate, sexine semitectate, subreticulate exine, sexine thicker than nexine.
8g.	*Caesalpinia pulcherrima*	Caesalpinaceae-Caesalpinioideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, sub spheroidal prolate shape, tricolporate, ora circular, synaperturate, reticulate exine, semitectate.
8h.	*Caesalpinia bonduc*	Caesalpinaceae-Caesalpinioideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, sub spheroidal prolate shape, tricolporate, ora lolongate, synaperturate, reticulate exine, semitectate.
9a.	*Tridax procumbens*	Asteraceae-Asteroideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, prolate- spheroidal, polypantoporate and spinulate, spines short with pointed ends.
9b.	*M. micrantha*	Asteraceae-Asteroideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, prolate- spheroidal shape, trizonocolporate, spinulate, spines short, endocolpium lalongate, sexine thicker than nexine.
9c.	*Ageratum conyzoides*	Asteraceae-Asteroideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, prolate- spheroidal, polypantoporate endocolpium lalongate, and spinulate, spines are with sharp ends, sexine thicker than nexine.
9d.	*Bidens pilosa*	Asteraceae-Asteroideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, prolate- spheroidal shape, trizonocolporate, longicolpate, endocolpium sigmoid type, sexine thicker than nexine.
9f.	*Synedrella nodiflora*	Asteraceae-Asteroideae	Pollen grains are monads, circular amb, prolate- spheroidal, polycolporate and spinate, spines short with pointed ends.

9h.	*Boerrhavia diffusa*	Nyctaginaceae	Pollen grains are monads, radially symmetrical, spheroidal, pantoporate, tectum tubuliferous and spinulose, sexine thicker than nexine.
9i.	*Terminalia arjuna*	Combretaceae	Pollen grains monads, isopolar, radially symmetrical, circular amb, spheroidal shape, trizonocolporate alternating with pseudocolpi, side tapering to acuminate tips, ora lalongate, exine psilate, sexine thicker than nexine.
10a.	*Psidium guajava*	Myrtaceae-Myrtoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb with concave sides, oblate shape, parasyncolpate, tricolporate, angulaperturate, psilate ornamentation, sexine thicker than nexine.
10b.	*P. araca*	Myrtaceae-Myrtoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, oblate shape, parasyncolpate, tricolporate, angulaperturate, psilate ornamentation, sexine thicker than nexine.
10d.	*Syzygium cumini*	Myrtaceae-Myrtoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, oblate shape, parasyncolpate, apocolpial field present, tricolporate, angulaperturate, psilate ornamentation, sexine thicker than nexine.
10f.	*Callistemon citrinus*	Myrtaceae-Myrtoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, pollen sides slightly convex, oblate shape, parasyncolpate, angulaperturate, tricolporate, apocolpial field psilate, exine psilate, sexine thicker than nexine.
10h.	*Swietenia mahagoni*	Meliaceae-Swietenioideae	Pollen grain monads, isopolar, bilaterally symmetrical, circular amb, spheroidal shape, tetracolporate, ora lolongate, psilate, sexine as thick as nexine.
11a.	*Alternanthera sessilis*	Amaranthaceae-Gomphrenoideae	Pollen grain monad, isopolar, bilaterally symmetrical, dodecahedron shape, pantoporate, 12 pores, metareticulum ornamentation.
11b.	*Amaranthus spinosus*	Amaranthaceae-Amaranthoideae	Pollen grains are monads, apolar, radially symmetry, spheroidal, pantoporate, small pores without operculum, exine sparsely scabrate, sexine is thicker than nexine.

11c.	*A. viridis*	Amaranthaceae-Amaranthoideae	Pollen grains are monads, apolar, radially symmetry, spheroidal, pantoporate, pore plate densely scabrate, small pores without operculum, sexine thicker than nexine.
11d.	*A. hybridus*	Amaranthaceae-Amaranthoideae	Pollen grains are monads, apolar, radially symmetry, spheroidal, pantoporate, pores larger, interpore distance is small, sparsely scabrate, sexine thicker than nexine.
11e.	*Ceiba pentandra*	Malvaceae-Bombacoideae	Pollen grains are monads, isopolar, bilateral symmetry, triangular amb, subprolate, tetrazonocolporate, ora lolongate, reticulate and heterobrochate sexine, sexine thicker than nexine.
11g.	*P. foetida*	Passifloraceae-Passsofloroideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal shape, 6 syncolpate aperture, heteroreticulate exine with columellate and simple muri, fewer bacula in lumen.
11h.	*Passiflora edulis*	Passifloraceae-Passsofloroideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal shape, 6 syncolpate aperture, heteroreticulate exine with columellate and simple muri, many bacula in lumen.
12a.	*Macaranga peltata*	Euphorbiaceae-	Pollen grain monads, isopolar, bilaterally symmetrical, circular amb, spheroidal, tricolporate, ora lalongate, scabrate surface,
12d.	*Euphorbia hetrophylla*	Euphorbiaceae-Euphorbioideae	Pollen grains are monads, isopolar, bilaterically symmetrical, circular amb, spheroidal shape, tricolpate, reticulate exine, sexine thicker than nexine.
12e.	*Poinsettia pulcherrima*	Euphorbiaceae-Euphorbioideae	Pollen grains are monads, isopolar, bilaterically symmetrical, circular amb, spheroidal shape, tricolpate, reticulate exine, free standing collumellae, sexine thicker than nexine.
12i.	*Phyllanthus urinaria*	Phyllanthaceae-Phyllanthoideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, prolate shape, pentacolpate, colpi narrow, bireticulate exine, sexine thicker than nexine.
13a.	*J. procumbens*	Acanthaceae-Acanthoideae	Pollen grains are monads, isopolar, elliptic amb, prolate shape, dicolporate with 4 pseudocolpi, heteroaperturate, ora lalongate, reticulate, perforate, microreticulate exine patterns.

13b.	*Justicia gendarussa*	Acanthaceae-Acanthoideae	Pollen grains are monads, isopolar, subtriangular amb, prolate shape, tricolporate with 6 pseudocolpi, heteroaperturate, ora lalongate and reticulate perforate exine, sexine tegillate, sexine thicker than nexine.
13c.	*J. adhatoda*	Acanthaceae-Acanthoideae	Pollen grains are monads, isopolar, oblongate amb, prolate shape, dicolporate with 4 pseudocolpi, ora circular distinct, reticulate microreticulate areolate exine, sexine thicker than nexine.
13d.	*J. betonica*	Acanthaceae-Acanthoideae	Pollen grains are monads, isopolar, circular amb, prolate shape, tricolporate with 6 pseudocolpi, heteroaperturate ora lalongate and reticulate perforate exine.
13e.	*Scurrula parasitica*	Loranthaceae-Loranthoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, trilobite triangular amb, oblate shape, tricolpate, syncolpate, distinct margo at apex, granulate exine, nexine thickened at polar area.
13f.	*Dendrophthoe falcata*	Loranthaceae-Loranthoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, trilobate to concave triangular amb, distinctly oblate shape, syn tricolpate, margo distinct, nano verrucate exine.
14a.	*Coccinia grandis*	Cucurbitaceae-Cucurbitoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate spheroidal, trizonocolporate, ora lolongate, retipilate, sexine integillate, sexine thick as nexine.
14b.	*Momordica charantia*	Cucurbitaceae-Cucurbitoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal, trizonocolporate, ora circular to lolongate, retipilate, sexine integillate, sexine twice thicker than nexine.
14c.	*Lagenaria siceraria*	Cucurbitaceae-Cucurbitoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate spheroidal to subprolate, trizonocolporate, ora lalongate, reticulate, heterobrochate, sexine tegillate, sexine thick as nexine.
14d.	*Benincasa hispida*	Cucurbitaceae-Cucurbitoideae	Pollen grains are monad, isopolar, bilaterally symmetrical, circular amb, spheroidal shape, tricolporate, reticulate exine, heterobroochate sexine thicker than nexine.
14e.	*Trichosanthes tricuspidata*	Cucurbitaceae-Cucurbitoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal, triporate, reticulate ornamentation, muri wide.

14i.	*Sesamum malabaricum*	Pedaliaceae-Asternae	Pollen grains are monads, isopolar, radially symmetrical, sub oblate shape, zonocolpate, polycolpate (12 colpate), circumaperturate, retipilate-verrucate, sexine thicker than nexine.
15a.	*Ixora cocinea*	Rubiaceae-Ixoroideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal shape, tricolporate, microreticulate, eutectate, sexine thicker than nexine.
15b.	*Morinda citrifolia*	Rubiaceae-Rubioideae	Pollen grains are monad, isopolar, bilaterally symmetrical, subtriangular amb, oblate spheroidal shape, tricolporate, ora lolongate, reticulate exine.
15c.	*Mitracarpus hirtus*	Rubiaceae-Rubioideae	Pollen grains are monads, isopolar, circular amb, suboblate, hexazonocolpate, colpi long and narrow, microreticulate, heterobrochate, sexine thicker than nexine.
15f.	*Evolvulus glomeratus*	Convolvulaceae-Convolvuloideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, spheroidal shape and pentacolpate, and circumaperturate, colpi short with slit like opening arranged in geometric pattern, perforate-microreticulate exine and sexine thick as nexine.
15g.	*Merremia vitifolia*	Convolvulaceae-Dichondroideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate spheroidal, microreticulate exine, microechinate, spines with blunt ends.
15h.	*Hewittia malabarica*	Convolvulaceae-Convolvuloideae	Pollen grains are monads, isopolar, radially symmetrical, prolate spheroidal shape, periporate, spines with blunt apices, irregularly arranged and exine microreticulate granulate.
15i.	*Jacquemontia pentanthos*	Convolvulaceae-Dichondroideae	Pollen grains are monads, isopolar, circular amb, spheroidal shape, pentacolpate, perforate-microechinate, tectate exine and sexine thicker than nexine.
16c.	*Ocimum gratissimum*	Lamiaceae-Nepetoideae	Pollen grains are monads, isopolar, radially symmetrical, circular amb, subspheroidal, hexazonocolpate, circumaperturate, megareticulate tectum, sexine thicker than nexine.
16d.	*Vitex negundo*	Lamiaceae-Viticoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal shape, tricolpate, tectate perforate exine.

16e.	*Clerodendron thomsonae*	Lamiaceae-Ajugoideae	Pollen grains are monads, heteropolar, radially symmetrical, circular amb, spheroidal shape, tricolpate, microechinate perforate exine, eutectate sexine, sexine as thick as nexine.
16f.	*Annona muricata*	Annonaceae-Annonoideae	Pollen grains are rhomboidal tetrad, planar, heteropolar, bilaterally symmetrical, ellipsoidal amb, oblate shape, monosulcate, reticulate foveolate exine.
16g.	*A. squamosa*	Annonaceae-Annonoideae	Pollen grains are rhomboidal tetrad, planar, isopolar, bilaterally symmetrical, inaperturate, foveolate exine, sexine as thick as nexine.
16h.	*Melastoma malabathricum*	Melastomaceae-Melastomatoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, prolate spheroidal, trizonocolporate, colpi ends tapering obtuse, ora circular, psilate exine, subsidiary colpi assymetrical.
17a.	*Nymphoides indica*	Menyanthaceae-Menyanthoideae	Pollen grains are monads, isopolar, triangular amb, oblate shape, tricolpate, parasyncolpate, rugulate exine, sexine thicker than nexine.
17c.	*Careya arborea*	Lecythidaceae-Planchonioideae	Pollen grains are monad, trilobed amb, spheroidal shape, trizonocolporate, syncolpate, colpi membrane has two rows of gemmate- verrucose sculpturing elements, ora lolongate and exine plated or reticulate.
17d.	*Calophyllum inophyllum*	Calophyllaceae-Callophylloideae	Pollen grains are monads, isopolar, radially symmetry, triangular amb, tricolporate, ora lolongate, reticulate exine, sexine as thick as nexine.
17e.	*Tabernaemontana gamblei*	Apocynaceae-Rauvolfoideae	Pollen grains are monads, isopolar, circular amb, prolate shape, tetrazonocolporate, brevicolpate, psilate exine.
17f.	*T. diverticata*	Apocynaceae-Rauvolfoideae	Pollen grains are monads, isopolar, circular amb, prolate shape, trizonocolporate, brevicolpate, psilate exine.
17i.	*Trianthema portulacastrum*	Aizoceae-Sesuvioideae	Pollen grains are monads, isopolar, radially symmetrical, subcircular amb, subprolate, tricolpate and sparsely punctate exine.

8c.	*Areca sp.*	Arecaceae-Arecoideae	Pollen grains are monad, amb elliptical, monocolpate, pantocolpate, sexine reticulate, heterobrochate, muri simplibaculate, sexine thicker than nexine.
9e.	*Chromolaena sp.*	Asteraceae-Asteroideae	Pollen grains are monads, circular amb, oblate- spheroidal, polycolporate ans spinate, spines short with pointed ends.
10c.	*Eucalyptus sp.*	Myrtaceae-Myrtoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, oblate shape, parasyncolpate, tricolporate, angulaperturate, psilate ornamentation, sexine thicker than nexine.
10e.	*Syzygium sp.*	Myrtaceae-Myrtoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, amb weakly convex, oblate shape, parasyncolpate, tricolporate, angulaperturate, psilate ornamentation, sexine thicker than nexine.
12b.	*Croton* sp.	Euphorbiaceae-Crotonoideae	Pollen grains are monads, apolar, spherical shaped, inaperturate, exine ornamented with pila in crotonoid pattern, 5-6 sub triangular- triangular psilate clava arranged in units.
12c.	*Jatropha sp.*	Euphorbiaceae-Crotonoideae	Pollen grains are monads, isopolar, radially symmetrical, spheroidal, inaperturate, exine heavily sculptured crotonoid pattern, sparsely gemmate/ clavate with very few muri.
12f.	*Manihot sp.*	Euphorbiaceae-Crotonoideae	Pollen grains are monads, apolar, spherical shaped, inaperturate, exine ornamented with pila in crotonoid pattern, 6-7 sub triangular psilate clava arranged in units.
12g.	*Glochidion sp.*1	Phyllanthaceae-Phyllanthoideae	Pollen grains are monads, isopolar, circular amb, spheroidal shape, tetracolporate, reticulate exine, sexine tectate, sexine as thick as nexine.
12h.	*Glochidion sp.*2	Phyllanthaceae-Phyllanthoideae	Pollen grains are monads, isopolar, circular amb, spheroidal shape, pentacolporate, reticulate exine, sexine tectate, sexine as thick as nexine.
13g.	*Macrosolen sp.*	Loranthaceae-Loranthoideae	Pollen grains are monads, elliptic equatorial limb, oblate shape, syn tricolpate, broadly rounded apices and margo indistinct, micro verrucate perforate.
13h.	*Helixanthera sp.*	Loranthaceae-Loranthoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, concave triangular amb, distinctly oblate shape, syn tricolpate, margo indistinct, granulate to verrucate exine, nexine thickened at intercolpi.

14f.	*Trichosanthes sp.*	Cucurbitaceae-Cucurbitoideae	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, oblate spheroidal, tricolporate, reticulate ornamentation, muri wide.
15d.	*Spermacoce sp. 1*	Rubiaceae-Rubioideae	Pollen grains monads, isopolar, radially symmetrical, circular amb, oblate shape, polycolpate (7 colpate), zonocolpate, brevicoplate, distinctly reticulate exine, heterobrochate, sexine thicker than nexine.
15e.	*Spermacoce sp. 2*	Rubiaceae-Rubioideae	Pollen grains monads, isopolar, radially symmetrical, circular amb, oblate shape, polycolpate (14 colpate), zonocolpate, brevicoplate, distinctly reticulate exine, heterobrochate, sexine thicker than nexine.
16a.	*Citrus sp.*	Rutaceae-Aurantioideae	Pollen grains are monad, isopolar, radially symmetrical, circular amb, oblate spheroidal, tetracolporate, circulaperturate, oralalongate, exine reticulate,
17b.	*Lilium sp.*	Liliaceae-Lilioideae	Pollen grains are monads, heteropolar, ellipsoidal amb, boat shaped, prolate, monosulcate, sulcus reaching the ends of grains, semitectate, macroreticulate, heterobrochate, wide, compound, simplicollumellate muri.
8e.	Arecaceae sp.1	Arecaceae	Pollen grains are monads, heteropolar, bilaterally symmetrical, monocolpate, longicolpate, colpi with sharp ends, baculate exine.
8f.	Arecaceae sp. 2	Arecaceae	Pollen grains monads, heteropolar, monocolpate, longicolpate, verrucate exine.
9g.	Asteraceae sp. 1	Asteraceae	Pollen grains are monads, radially symmetrical, spheroidal shape, tricolporate, spinate, spines long with thick walls
10g.	Myrtaceae sp. 1	Myrtaceae	Pollen grains are monads, isopolar, bilaterally symmetrical, tri colporate, angulaperturate, sexine thinner than nexine, microreticulate exine.
11f.	Malvaceae sp. 1	Malvaceae	Pollen grains in monads, pantoporate, echinate, microreticulate perforate exine, sexine almost thick as nexine.

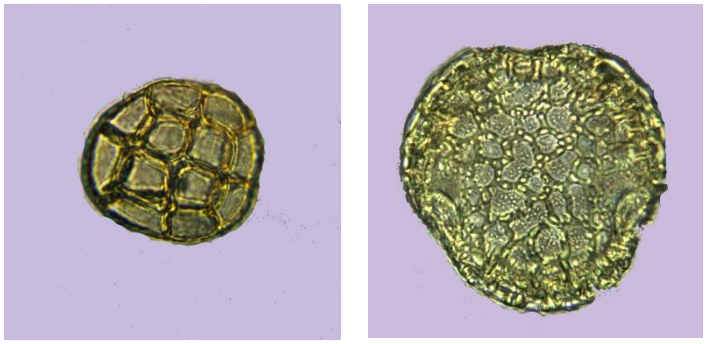
14g.	Poaceae sp. 1	Poaceae	Pollen grains monads, apolar, spheroidal, monoporate, areolate and scabrate, sexine thick as nexine.
14h.	Poaceae sp. 2	Poaceae	Pollen grains monads, apolar, spheroidal, monoporate, medium scabrate, sexine thick as nexine.
16b.	Rutaceae sp. 1	Rutaceae	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, prolate spheroidal, tricolporate, angulaperturate, tectate reticulate exine.
17g.	Caryophyllaceae sp.1	Caryophyllaceae	Pollen grains are monads, isopolar, radially symmetrical, spheroidal shaped, polypantoporate, tectum microperforate, microechinate.
17h.	Caryophyllaceae sp. 2	Caryophyllaceae	Pollen grains are monads, isopolar, radially symmetrical, spheroidal shaped, polypantoporate, microechinate.
18a.	Pollen type 1	--	Pollen grain monad, isopolar, bilaterally symmetrical, sub-triangular amb, tricolporate, angulaperturate, sunken colpori, reticulate exine, sexine almost thick as nexine.
18b.	Pollen type 2	--	Pollen grain monad, isopolar, radially symmetrical, circular amb, oblate-spheroidal shape, triporate, pore circular, exine striate.
18c.	Pollen type 3	--	Pollen grains monads, isolpolar, bilaterally symmetrical, circular amb, prolate- spheroidal shape, tricolporate, sriato-reticulate exine.
18d.	Pollen type 4	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate- spheroidal shape, tricolpate, exine perforate-microreticulate, sexine thinner than nexine.
18e.	Pollen type 5	--	Pollen grain monad, isopolar, bilaterally symmetrical, circular amb, oblate to oblate spheroidal shape, tricolpate, areolate-microreticulate exine, sexine thicker than nexine.
18f.	Pollen type 6	--	Pollen grains are monads, isopolar, bilaterally symmetrical, sub triangular amb, prolate to prolate spheroidal shape, tricolporate, angulaperturate, psilate exine.
18g.	Pollen type 7	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate shape, tricolpate, longicolpi, reticulate exine.

18h.	Pollen type 8	--	Pollen grains are monads, heteropolar, bilaterally symmetrical, circular amb, spinulose-echinate exine.
18i.	Pollen type 9	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, sub prolate to prolate spheroidal shape, tricolporate, longicolpate, exine reticulate.
18j.	Pollen type 10	--	Pollen grain monad, isopolar, radially symmetrical, circular amb, spheroidal shape, tricolpate, demicolpate, reticulate exine.
18k.	Pollen type 11	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate-spheroidal shape, tricolpate, demicolpate, areolate- reticulate exine,
18l.	Pollen type 12	--	Pollen grains are monads, bilaterally symmetrical, circular amb, tricolpate, sexine thicker than nexine.
18m.	Pollen type 13	--	Pollen grains are monads, apolar, radially symmetrical, circular amb, spheroidal shape, pantoporate, pilate exine.
18n.	Pollen type 14	--	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, triporate, fossulate- areolate exine.
18o.	Pollen type 15	--	Pollen grains are monads, isopolar, bilaterally symmetrical, prolate shape, tricolpate, sexine thicker than nexine.
18p.	Pollen type 16	--	Pollen grains are monads, isopolar, bilaterally symmetrical, triangular amb, tricolporate, angulaperturate, colpori sunken, foveolate- fossulate exine, sexine thinner than nexine.
18q.	Pollen type 17	--	Pollen grains are monads, isopolar and bilaterally symmetrical, reticulate exine.
18r.	Pollen type 18	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, prolate spheroidal, tricolporate, psilate exine.
18s.	Pollen type 19	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, tricolpate, faintly foveolate exine.

18t.	Pollen type 20	--	Pollen grains are monads, isopolar, bilaterally symmetrical, sub triangular amb, prolate spheroidal shape, tricolpate, angulaperturate, striate exine, sexine thicker than nexine.
18u.	Pollen type 21	--	Pollen grains are monads, isopolar, bilaterally symmetrical, circular amb, sub spheroidal shape, tricolpate and exine striate.
18v.	Pollen type 22	--	Pollen grains are monads, isopolar, radially symmetrical, circular amb, spheroidal shape, sexine tectate, reticulate exine.

a. **b.** **c.**

d. **e.** **f.**



g. **h.**

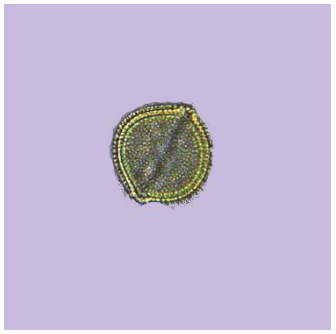
Plate 7. Light micrographs of pollen of family Fabaceae a. *Mimosa pudica* b. *M. diplotricha* c. *Milletia pinnata* d. *Peltophorum pterocarpum* e. *Acacia mangium* f. *Acacia latronum* g. *Acacia catehu* h. *Delonix reaia*

a.

b.

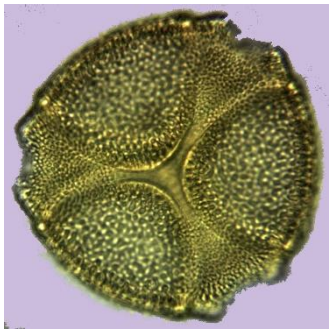
c.

d.

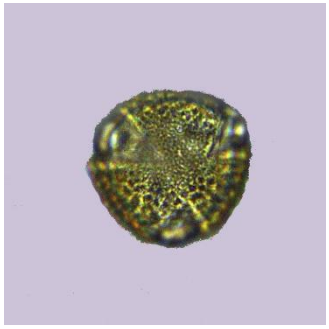


e.

f.

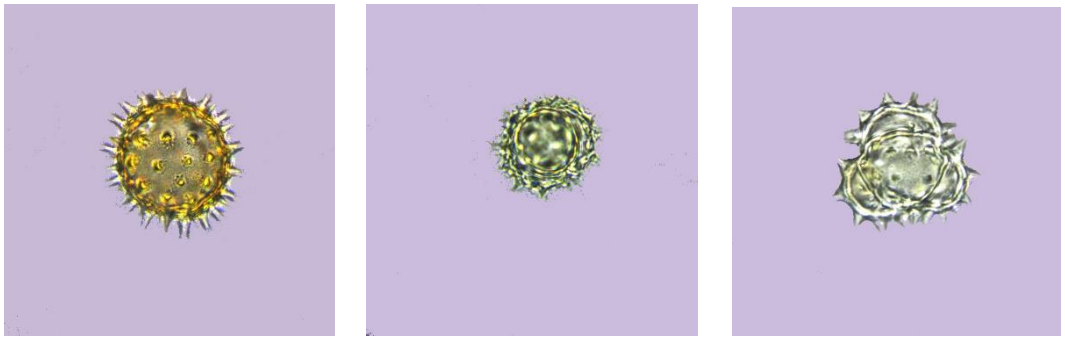


g.

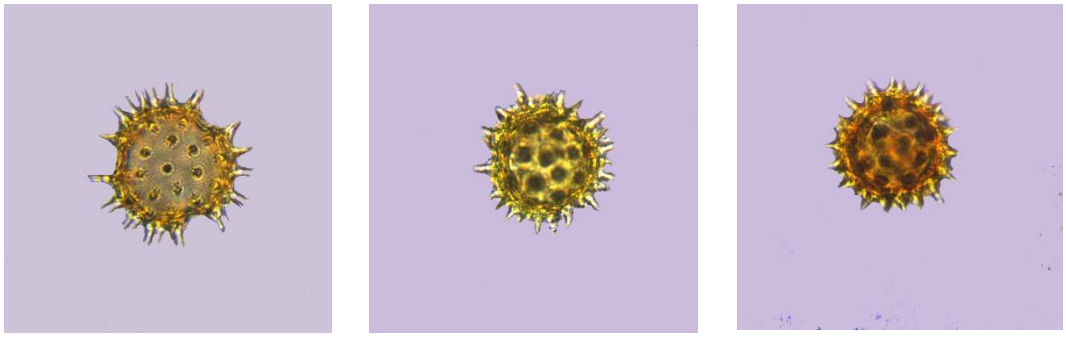


h.

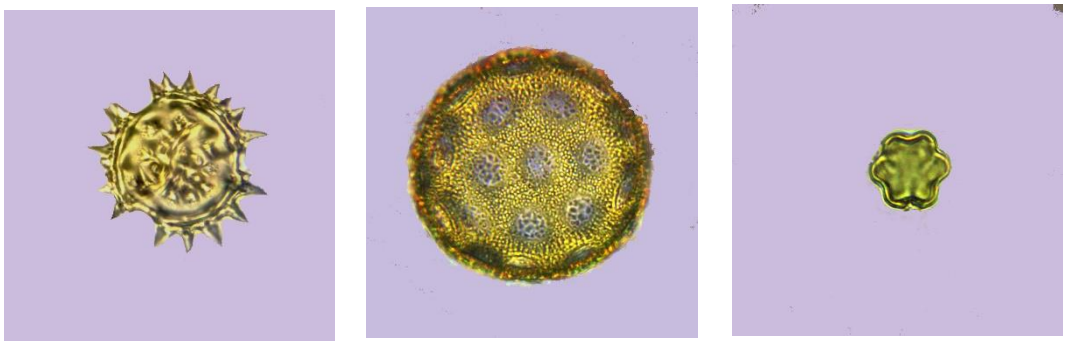
Plate 8. Light micrographs of pollen of family Arecaceae a. *Cocos nucifera* b. *Borassus flabellifer* c. *Areca* sp. d. *Elaeis guineensis* e. Arecaceae sp. 1 f. Arecaceae sp. 2 Caesalpiniaceae g. *Caesalpinia pulcherrima* h. *Caesalpinia bonduc*



a. **b.** **c.**

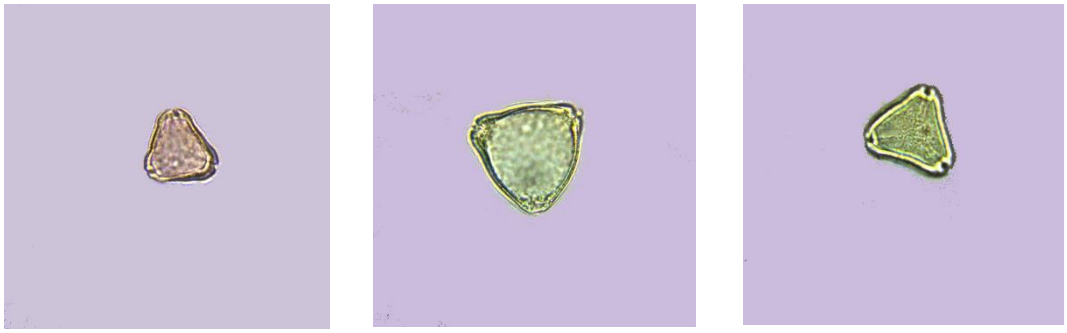


d. **e.** **f.**



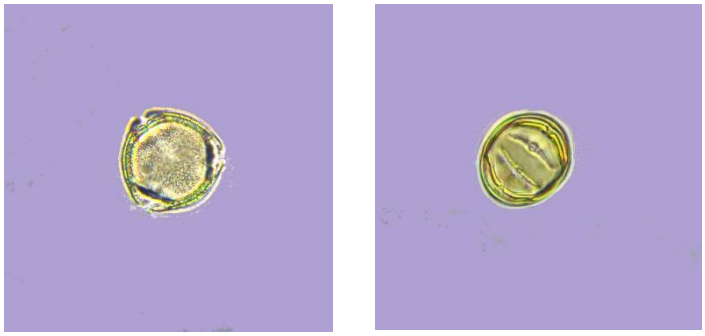
g. **h.** **i.**

Plate 9. Light micrographs of pollen of family Asteraceae a. *Tridax procumbens* b. *Mikania micrantha* c. *Ageratum conyzoides* d. *Bidens pilosa* e. *Chromolaena* sp. f. *Synedrella nodiflora* g. Asteraceae sp. 1 Nyctaginaceae h. *Boerrhavia diffusa* Combretaceae i. *Terminalia arjuna*



a. **b.** **c.**

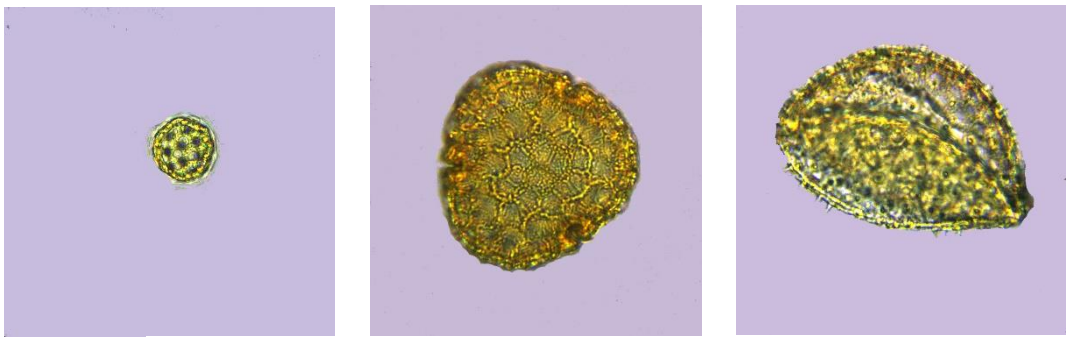
d. **e.** **f.**



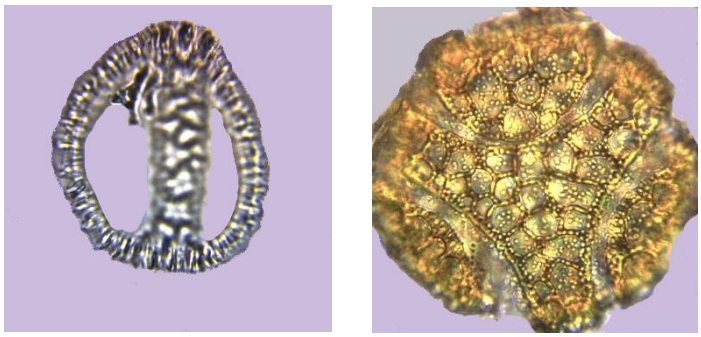
g. **h.**

Plate 10. Light micrographs of pollen of family Myrtaceae a. *Psidium guajava* b. *Psidium araca* c. *Eucalyptus* sp. d. *Syzygium cumini* e. *Syzygium* sp. f. *Callistemon citrinus* g. Myrtaceae sp. 1 Meliaceae h. *Sweitenia mahagoni*

a. **b.** **c.**



d. **e.** **f.**

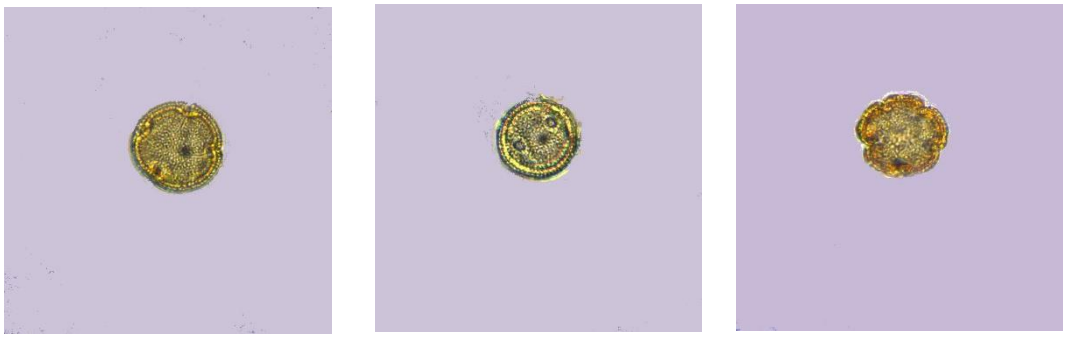


g. **h.**

Plate 11. Light micrographs of pollen of family Amaranthaceae a. *Alternanthera sessilis* b. *Amaranthus spinosus* c. *Amaranthus viridis* d. *Amaranthus hybridus* Malvaceae e. *Ceiba pentandra* f. Malvaceae sp. 1 Passifloraceae g. *Passiflora foetida* h. *Passiflora edulis*

a. **b.** **c.**

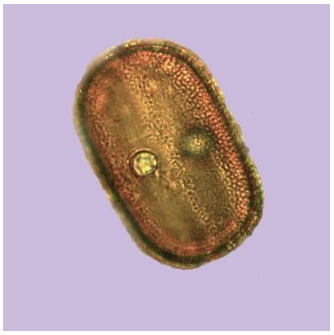
d. **e.** **f.**



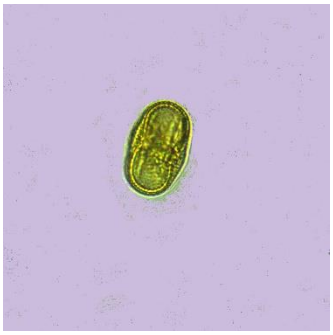
g. **h.** **i.**

Plate 12. Light micrographs of pollen of family Euphorbiaceae a. *Macaranga peltata* b. *Croton* sp. c. *Jatropha* sp. d. *Euphorbia heterophylla* e. *Poinsettia pulcherrima* f. *Manihot* sp. Phyllanthaceae g. *Glochidion* sp.1 h. *Glochidion* sp. 2 i. *Phyllanthus urinaria*

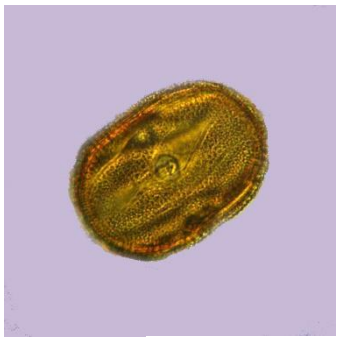
a.



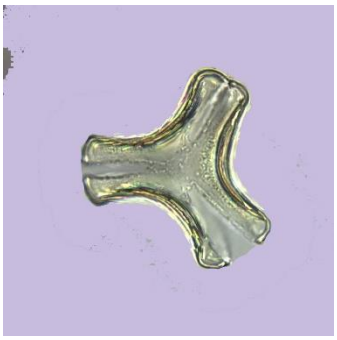
b.



c.



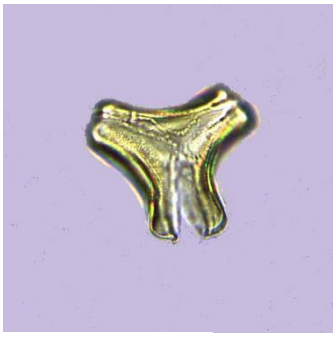
d.



e.

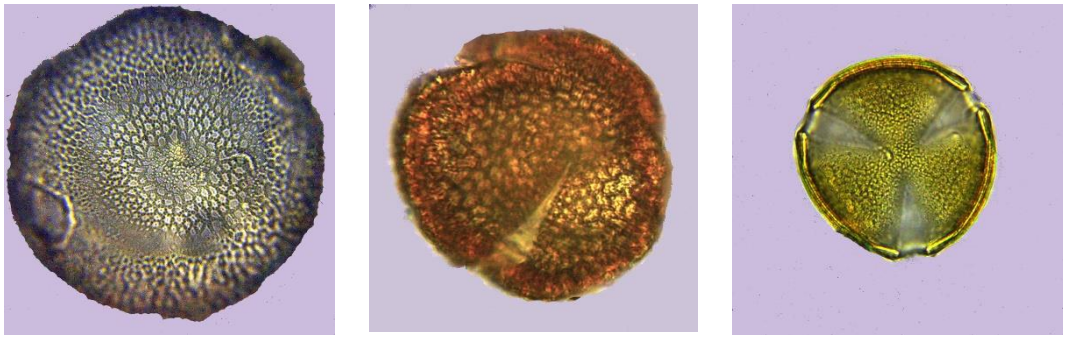
f.

g.

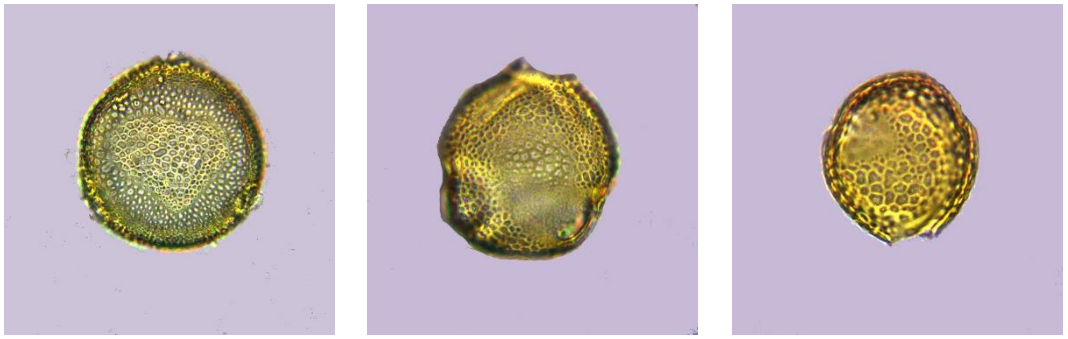


h.

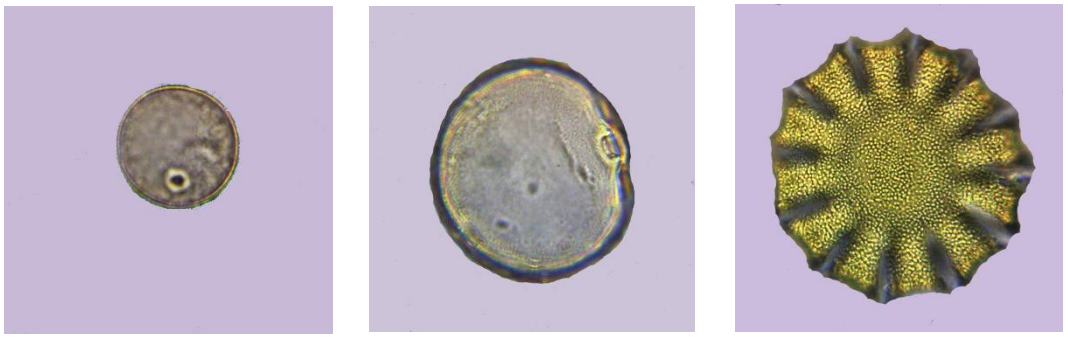
Plate 13 Light micrographs of pollen of family Acanthaceae a. ***Justicia procumbens*** **b.** ***Justicia gendarussa*** **c.** ***Justicia adhatoda*** **d.** ***Justicia betonica*** **Loranthaceae e.** ***Scurrula parasitica*** **f.** ***Dendrophthoe falcata*** **g.** ***Macrosolen*** **sp. h.** ***Helixanthera sp.***



a. **b.** **c.**



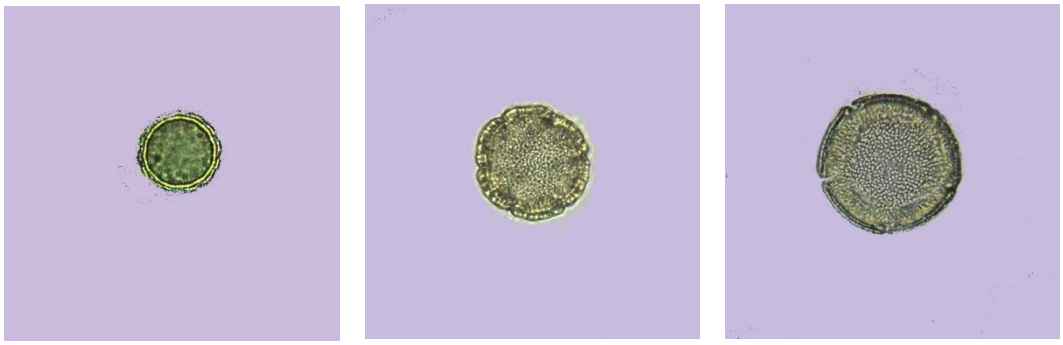
d. **e.** **f.**



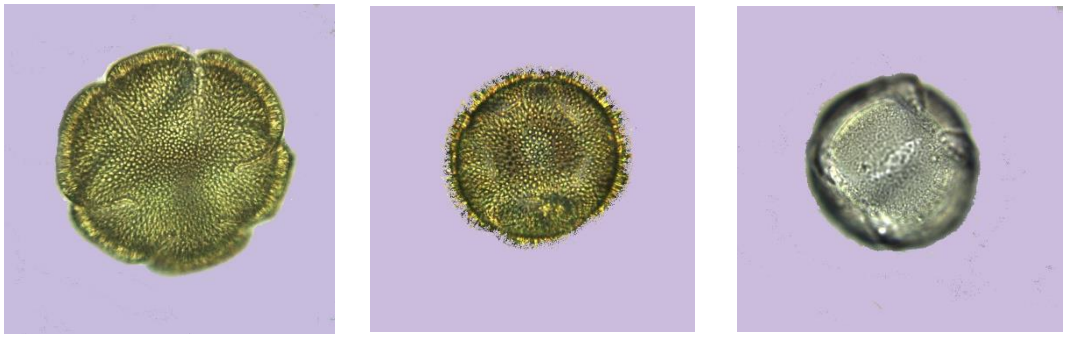
g. **h.** **i.**

Plate 14. Light micrographs of pollen of family Cucurbitaceae a. *Coccinia grandis* b. *Momordica charantia* c. *Lagenaria siceraria* d. *Benincasa hispida* e. *Trichosanthes tricupidata* f. *Trichosanthes sp.* Poaceae g. Poaceae sp. 1 h. Poaceae sp. 2 Pedaliaceae i. *Sesamum malabaricum*

a. **b.** **c.**

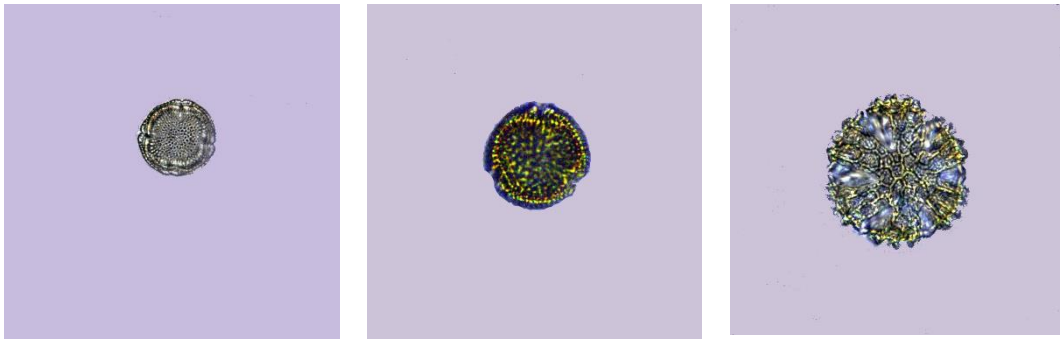


d. **e.** **f.**

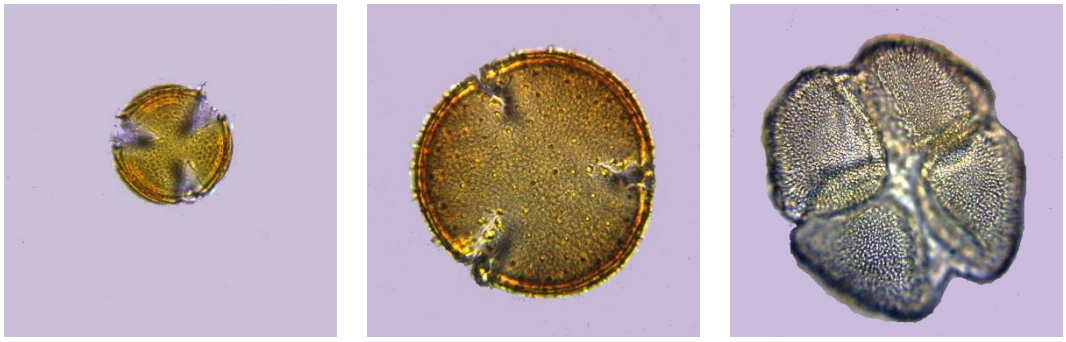


g. **h.** **i.**

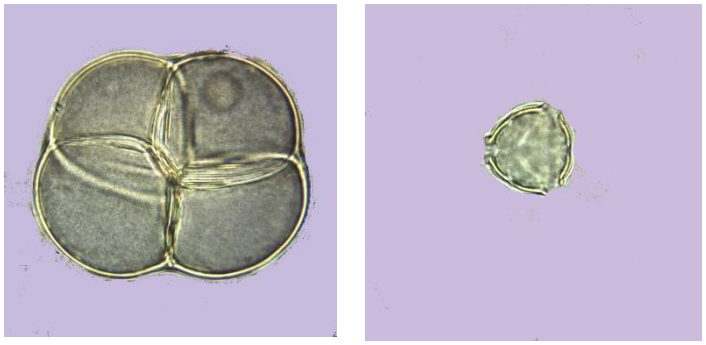
Plate 15. Light micrographs of pollen of family Rubiaceae a. *Ixora coccinea* b. *Morinda citrifolia* c. *Mitracarpus hirtus* d. *Spermacoce* sp. 1 e. *Spermacoce* sp. 2 Convolvulaceae f. *Evolvulus glomeratus* g. *Merremia vitifolia* h. *Hewittia malabarica* i. *Jacquemontia pentanthos*



a. **b.** **c.**



d. **e.** **f.**

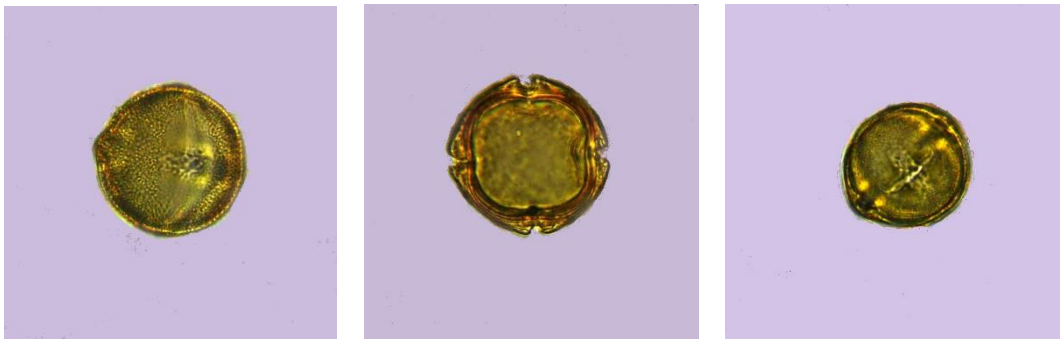


g.

h.

Plate 16. Light micrographs of pollen of family Rutaceae a. *Citrus* sp. b. Rutaceae sp. 1 Lamiaceae c. *Ocimum gratissimum* d. *Vitex negundo* e. *Clerodendron thomsonae* Annonaceae f. *Annona muricata* g. *Annona squamosa* Melastomaceae h. *Melastoma malabathricum*

a. **b.** **c.**

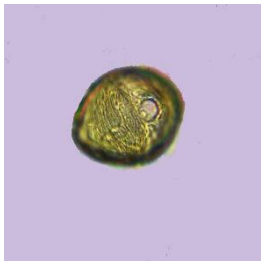


d. **e.** **f.**

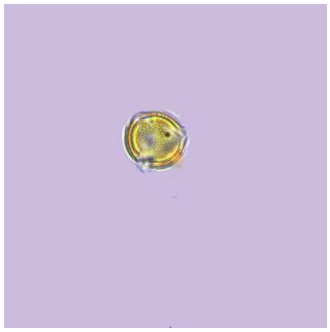
g. **h.** **i.**

Plate 17. Light micrographs of pollen of family Menyanthaceae a. *Nymphoides indica* Liliaceae b. *Lilium* sp. Lecythidaceae c. *Careya arborea* Calophyllaceae d. *Calophyllum inophyllum* Apocynaceae e. *Tabernaemontana gamblei* f. *Tabernaemontana diverticata* Caryophyllaceae g. Caryophyllaceae sp. 1 h. Caryophyllaceae sp. 2 Aizoaceae i. *Trianthema potulacastrum*

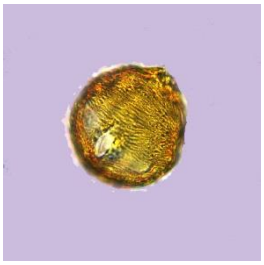
a.



b.

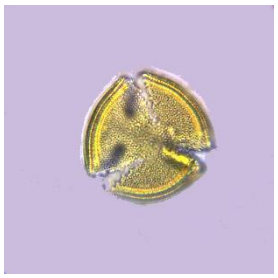


d.



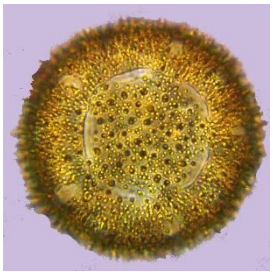
c.

f.



e.

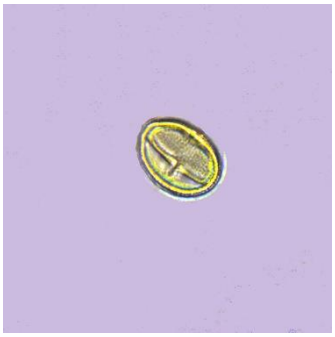
g.



h.

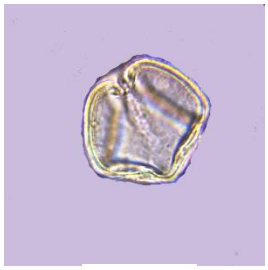
i.

j.

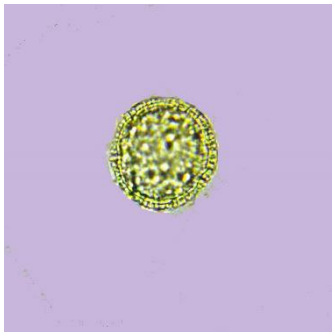


k.

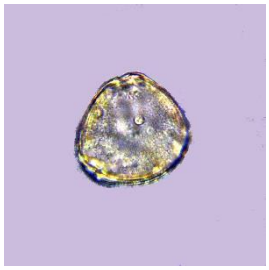
Plate 18. Light micrographs of unidentified pollen a. ***P. type 1*** **b.** ***P.type 2.*** **c.** ***P. type 3*** **d.** ***P. type 4*** **e.** ***P. type 5*** **f.** ***P. type 6*** **g.** ***P. type 7*** **h.** ***P. type 8*** **i.** ***P. type 9*** **j.** ***P. type 10*** **k.** ***P. type 11***



l.



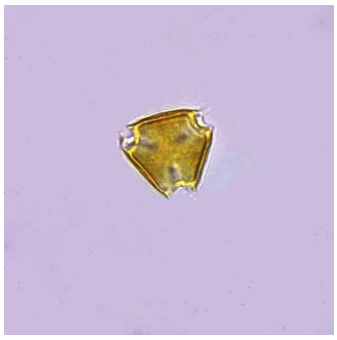
m.



n.

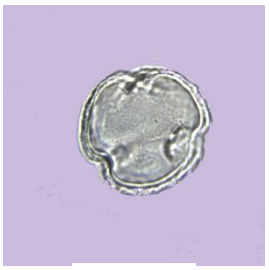
o.

q.

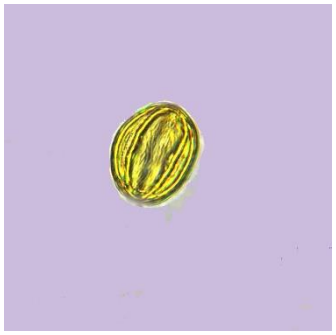


p.

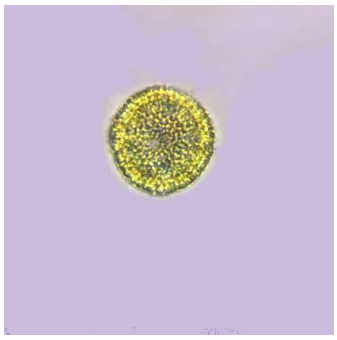
r.



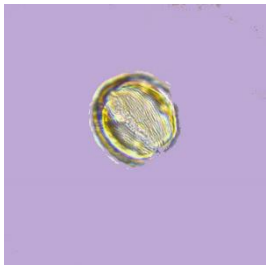
s.



t.



v.



u.

Plate 18 cont. Light micrographs of unidentified pollen l. ***P. type 12*** **m.** ***P.type 13.*** **n.** ***P. type 14*** **o.** ***P. type15*** **p.** ***P. type 16*** **q.** ***P. type 17*** **r.** ***P. type 18*** **s.** ***P. type 19*** **t.** ***P. type 20*** **u.** ***P. type 21*** **v.** ***P. type 22***

4.2.1 Distribution of pollen types in pollen loads during northeast monsoon season (October- December)

The predominant pollen types in northeast monsoon season included *C. nucifera*, *M. pudica*, *M. pinnata* and *M. peltata*. A total of 31 pollen types were recorded from the pollen loads (Table 4). The maximum number of pollen types were recorded from Tholicode (10), followed by Powdikonam (9). The locations Panacode, Vembayam, Peppara had eight pollen types each while seven pollen types were recorded from Perumpazhuthoor, Poovar, Panayamuttom and Kallara. The locations Kilimanoor, Kaviyode, Amboori, Perukavu and Chennanpara recorded six pollen types each. Five pollen types were recorded from Melvettoor and Machel and least number of pollen types were recorded from Kulathoor (3).

Distribution of *C. nucifera* in the predominant pollen class ranged from 55.66 to 86.9 per cent in three locations Machel, Poovar and Perukavu. Seven locations, namely Chennanpara, Tholicode, Melvettoor, Powdikonam, Thekkada, Kaviyode, and Kilimanoor showed *M. pudica* as predominant pollen class which ranged from 49.74 to 91.2 per cent. Two locations Panayamuttom and Perumpazhuthoor recorded *M. pinnata* (50.39 to 72.24 per cent) as predominant pollen type. *M. peltata* (51.68 per cent) and Pollen type 1 (88.87 per cent) were recorded as predominant from one location each, namely Panacode and Kulathoor respectively. *M. pudica* was also secondary pollen (23.28 to 35.84 per cent) in locations Perumpazhuthoor, Peppara, Kallara and as important minor pollen (11.43 to 11.46 per cent) in Machel and Panayamuttom. *C. nucifera* was found to be secondary pollen in Amboori (26.31 per cent) whereas in locations Kilimanoor, Panacode, Perumpazhuthoor, Kulathoor, Thekkada, Powdikonam, Tholicode and Chennanpara it was important minor pollen (14.21 to 3.32 per cent). *M. pinnata* was recorded as secondary pollen in Amboori (19.29 per cent) Peppara (33.25 per cent) and Kallara (29.62 per cent) while as important minor pollen (8.17 to 3.03 per cent) in locations Kaviyode, Poovar, Machel, Thekkada, Powdikonam, Melvettoor and Tholicode. Apart from these, pollen types included in both secondary and important minor pollen were *M. diplotricha* (26.9 to 4.23 per cent), *M. micrantha*

Table 4. Distribution of pollen type classes in pollen loads in northeast monsoon season (October-December)

Locations	**Pollen type classes and percentage**			
	Predominant pollen	**Secondary pollen**	**Important minor pollen**	**Minor pollen**
Kilimanoor	*M. pudica* (66.44)	--	*C. nucifera* (13.31)	*M. peltata*(3.67) *Areca* sp. (2.78) *M. pinnata* (1.01) Pollen type 4 (1.81)
Panacode	*M. peltata*.(51.68)	--	*C. nucifera* (14.21) *Areca* sp. (11.88) *M. malabathricum* (9.04)	*M. pudica* (2.84) Pollen type 9 (2.07) *T. procumbens* (1.8) *M. diplotricha* (0.51)
Kaviyode	*M. pudica* (65.24)	*M. micrantha* (29.77)	*M. pinnata* (3.63)	*T. procumbens* (0.32) *A. viridis* (0.32) *P. pterocarpum* (0.16)
Amboori	--	*C. nucifera* (26.31) *P. pterocarpum* (27.19) *Areca* sp. (21.9) *M. pinnata* (19.29)	*T. procumbens* (3.94)	*D. regia* (0.44)
Perumpazhuthoor	*M. pinnata* (50.39)	*M. pudica* (23.28)	*C. nucifera* (11.32) *M. peltata*(3.6)	Pollen type 10 (1.07) *E. glomeratus* (1.9) Pollen type 6 (2.75)

Kulathoor	Pollen type 1 (88.87)	--	*C. nucifera* (7.79) *B. flabellife*r (3.68)	--
Poovar	*C. nucifera* (55.66)	*B. flabellife*r (29.61)	*M. pinnata* (5.32) Pollen type 1 (7.4)	*M. pudica* (0.69) *S. parasitica* (0.48) *C. citrinus* (0.46)
Machel	*C. nucifera* (82.8)	--	*M. pudica* (11.46) *M. pinnata* (5.09)	*P. pterocarpum* (0.21) Pollen type 11 (0.42)
Thekkada	*M. pudica* (73.23)	--	*C. nucifera* (3.32) *M. pinnata* (4.37) *A. conyzoides* (8.74)	*M. diplotricha* (1.38) Pollen type 10 (0.57) *A. latronum* (0.04) *P. foetida* (0.04)
Powdikonam	*M. pudica* (66.27)	--	*C. nucifera* (8.82) *M. diplotricha* (12.01) *M. pinnata* (3.03)	*Eucalyptus* sp. (1.59) Pollen type 2 (1.88) *Areca* sp. (1.15) *A. conyzoides* (1.15) *J. procumbens* (1.44)
Panayamuttom	*M. pinnata* (72.24)	--	*M. pudica* (11.43) *T. procumbens* (3.96)	*J. gendarussa* (2.36) *D. falcata* (2.26) *C. nucifera* (2.64) *M. peltata* (1.79)
Melvettoor	*M. pudica* (91.2)	--	*M. pinnata* (3.25)	*C. nucifera* (0.32) *T. arjuna* (1.49)

				Pollen type 6 (1.04)
Perukavu	*C. nucifera* (86.9)	--	*T. procumbens* (7.14)	Pollen type 9 (2.38) *Areca* sp. (1.19) *A. sessilis* (1.19) *M. pudica* (1.19)
Peppara	--	*M. pinnata* (33.25) *M. pudica* (25.67)	*M. micrantha* (14.64) *A. viridis* (5.6) Pollen type 11 (4.98)	Pollen type 13 (2.26) *M. peltata* (1.1) *P. araca* (1.09)
Kallara	--	*M. pinnata* (29.62) *M. pudica* (35.84)	Arecaceae sp. 1 (15.61) *M. diplotricha* (4.23) *P. araca* (4.36)	*C. nucifera* (2.38) *A. conyzoides* (0.53)
Tholicode	*M. pudica* (49.74)	*M. peltata*(24.58)	*C. nucifera* (8.24) *M. pinnata* (8.17) *Areca* sp. (5.13)	Pollen type 6 (1.22) *A. hybridus* (0.86) *B. flabellifer* (0.43) *T. procumbens* (0.21) *T. arjuna* (0.8) *P. foetida* (0.07)
Chennanpara	*M. pudica* (58.07)	*M. diplotricha* (26.9)	*C. nucifera* (5.72) *M. peltata* (3.56)	*M. micrantha* (1.9) *A. sessilis* (1.84)

(29.77 to 14.64 per cent), *M. peltata* (24.58 to 3.25 per cent) *Areca* sp. (21.9 to 5.13 per cent) and *B. flabellifer* (29.61 to 3.68 per cent). *P. pterocarpum* (27.19 per cent) was reported in secondary pollen type class alone. Other important minor pollen types included *T. procumbens* (7.14 to 3.94 per cent), *A. conyzoides* (8.74 per cent), *A. viridis* (5.6 per cent), *M. malabathricum* (9.04 per cent), *P. araca* (4.36 per cent), Arecaceae sp. 1 (15.61 per cent), P. type 11 (4.98 per cent) and P. type 1 (7.1 per cent).

Locations Amboori, Peppara and Kallara had no predominant type of pollen whereas locations Kilimanoor, Kulathoor, Panacode, Machel, Thekkada, Powdikonam, Panayamuttom, Melvettoor and Perukavu did not record any secondary pollen type. All locations had important minor pollen types recorded.

4.2.2 Distribution of pollen types in honey samples during northeast monsoon season (October- December)

The predominant pollen type classes in honey samples in north-east monsoon season included *C. nucifera*, *M. pinnata*, *M. pudica*, *M. micrantha*, *M. peltata* and P. type 5. A total of 21 pollen types were recorded altogether from eighteen locations in the season. Maximum number of pollen types were observed in Powdikonam (7) followed by a count of six each in locations Peppara, Tholicode and Chennanpara. Locations Amboori, Aruvippuram and Panacode had four pollen types each whereas Kilimanoor and Poovar had three each which was the least (Table 5).

C. nucifera was the predominant pollen types in location Kilimanoor (87.5 per cent) and *M. pinnata* in location Poovar (83.07 per cent). Locations Powdikonam and Chennanpara recorded *M. pudica* (81.38 to 62.5 per cent) as predominant pollen type while in locations Peppara, Tholicode and Panacode it was *M. micrantha* (76.46 per cent), *M. peltata* (45.83 per cent) and P. type 5 (50.85 per cent) respectively. Although *C. nucifera* was a predominant pollen type, it was also recorded as a secondary pollen in Amboori (35.77 per cent) and Aruvippuram (31.82 per cent), as important minor pollen in Powdikonam (4.37 per cent) and a

Table 5. Distribution of pollen type classes in honey samples in northeast monsoon season (October-December)

Locations	**Pollen type classes and percentage**			
	Predominant pollen	**Secondary pollen**	**Important minor pollen**	**Minor pollen**
Kilimanoor	*C. nucifera* (87.5)	---	*T. procumbens* (3.9)	Asteraceae sp.1 (2.34)
Amboori	--	*C. nucifera* (35.77) *P. pterocarpum* (23.85)	*M. pinnata* (15.59) *M. peltata*(5)	--
Poovar	*M. pinnata* (83.07)	--	Pollen type 1 (12.33)	*C. nucifera* (2.29)
Aruvippuram	--	*C. nucifera* (31.82) *M. pinnata* (22.73) *T. procumbens* (27.27) *Areca* sp. (18.18)	--	--
Powdikonam	*M. pudica* (62.5)	--	*C. nucifera* (4.37) *Eucalyptus* sp. (6.88) *M. pinnata* (7.5) *Areca* sp. (5.62)	*M. diplotricha* (2.5) *J. procumbens* (1.88)
Peppara	*M. micrantha* (76.46)	*M. pinnata* (26.92) *M. peltata* (27.55)	Poaceae sp. 2 (11.17) *M. pudica* (11.53)	*T. procumbens* (2.56)
Tholicode	*M. peltata* (45.83)	*P. foetida* (16.6)	*T. procumbens* (8.3) Pollen type 9 (12.5) *M. pinnata* (4.16)	--

			C. pulcherrima(4.16)	
Chennanpara	*M. pudica* (81.38)	--	*M. diplotricha* (4.78) *M. micrantha* (3.72) *C. grandis* (3.19)	Pollen type 19 (3.12) *C. nucifera* (1.06)
Panacode	Pollen type 5 (50.85)	*Manihot* sp. (21.42) *P. foetida* (14.3)	*Areca* sp. (7.14)	--

minor pollen in Poovar (2.29 per cent) and Chennanpara (1.06 per cent). Similarly *M. pinnata* was also a secondary pollen in Aruvippuram (22.73 per cent) and Peppara (26.92 per cent) and important minor pollen in Amboori (15.59 per cent), Powdikonam (7.5 per cent) and Tholicode (4.16 per cent). *M. pudica* was also an important minor pollen in Peppara (11.53 per cent). *T. procumbens* was found a secondary pollen type in Aruvippuram (27.27 per cent) and important minor pollen in Kilimanoor (3.9 per cent) and Tholicode (8.3 per cent). Likewise *Areca* sp. was recorded a secondary pollen type in Aruvippuram (18.18 per cent) and important minor pollen in Panacode (7.14 per cent). The location Peppara (27.55 per cent) had *M. peltata* as secondary pollen while it was an important minor pollen in Amboori (5 per cent). Other secondary pollen types included *P. pterocarpum*, *P. foetida* and *Manihot* sp. *C. pulcherrima*, *Eucalyptus* sp., *M. micrantha*, *Coccinia grandis*, *M. diplotricha*, Poaceae sp. 2, P. type 1 and P. type 9 were important minor pollen types.

There was no predominant pollen type in Amboori and Aruvippuram. Meanwhile no secondary pollen type occurred in Kilimanoor, Poovar, Powdikonam and Chennanpara and no important minor pollen in location Aruvippuram.

4.2.3 Distribution of pollen types in pollen loads during dry season (January-May)

The distribution of pollen types identified in the pollen loads of dry season are recorded in Table 6. A total of 34 pollen types were identified in the season out of which most dominant pollen were of *C.nucifera*, *M. pudica*, *M. pinnata*, *D. regia*, *M. peltata*, P. type 3, P. type 2, P. type 22. Maximum number of pollen types occurred in location Kilimanoor (12) followed by Amboori (10). Nine pollen type was recorded from location Panayamuttom while eight types were observed in locations Aruvippuram, Peppara and Tholicode. Locations Poovar, Machel and Perukavu each was recorded with seven pollen types, Powdikonam, Melvettoor, Kallara and Chennanpara were recorded with six pollen types. Five pollen types

Table 6. Distribution of pollen type classes in pollen loads in dry season (January- May)

Locations	**Pollen type classes and percentage**			
	Predominant pollen	**Secondary pollen**	**Important minor pollen**	**Minor pollen**
Kilimanoor	--	Pollen type 1 (30.04)	*Areca* sp. (7.3) *M. pudica* (7.22) *M. pinnata* (9.03) Pollen type 7 (4.26) Pollen type 20 (8.53) *M. peltata* (7.71) *S. mahagoni* (4.92)	Poaceae sp. 1 (1.97) *T. arjuna* (2.62) Pollen type 5 (2.95) *C. nucifera* (1.81)
Panacode	*C. nucifera* (77.07)	--	*M. pudica* (7.73) *M. pinnata* (3.4) Pollen type 11 (7.5)	*P. pterocarpum* (1.93)
Amboori	--	*C. nucifera* (18.89) *A. sessilis* (32.79)	*P. pterocarpum* (6.63) *P. guajava* (5.25) *M. pudica* (13.14) *Areca* sp. (8.01) *M. diplotricha* (3.62)	*M. pinnata* (2.25) *A. conyzoides* (2.5) *M. peltata* (2.5)
Perumpazhuthoor	Pollen type 2 (60.55)	*C. nucifera* (17.46) Pollen type 5 (19.28)		*D. falcata* (1.31)
Kulathoor	--	*C. nucifera* (44.9) Pollen type 1 (43.15)	*M. pinnata* (10.35)	*B. flabellifer* (0.32)

Poovar	*M. peltata*(66.44)	*M. pinnata* (20.88)	Pollen type 1 (4.44)	*C. nucifera* (2.14) *B. flabellifer* (2.39) *S. mahagoni* (1.21) *P. guajava* (1.69)
Aruvippuram	*M. pinnata* (59.01)	--	*C. nucifera* (8.07) Pollen type 2 (10.58) Pollen type 3 (4.84) *A. sessilis* (3.05) *M. peltata* (3.05)	*Areca* sp. (1.52) *I. coccinea* (1.52)
Machel	*M. peltata* (46.94)	--	*C. nucifera* (14.89) *M. pinnata* (11.04) Myrtaceae sp. 1 (6.25) *M. pudica* (4.94) *Glochidion* sp. 2 (12.21)	*P. pterocarpum* (0.36)
Powdikonam	--	*C. nucifera* (37.89) *S. mahagoni* (39.8)	Pollen type 2 (12.10) Pollen type 14 (4.14)	*Areca* sp. (0.95) *A. spinosus* (0.95)
Panayamuttom	Pollen type 3 (46.96)	*A. spinosus* (18.31)	*C. nucifera* (7.3) *M. pinnata* (12.87) *M. peltata* (9.64)	*Glochidion* sp. 2 (1.98) Pollen type 11 (1.49) Pollen type 2 (2.5) Pollen type 4 (2.22)
Melvettoor	*C. nucifera* (63.37)	Pollen type 2 (17.19)	*M. pinnata* (7.32) *M. peltata*(7.32)	*C. inophyllum* (1.27) Pollen type 6 (1.6)

Perukavu	*M. pudica* (50.73)	*M. pinnata* (25.53)	*C. nucifera* (7.61) *A. conyzoides* (8.04) *M. peltata* (3.69)	*A. sessilis* (0.94) Pollen type 9 (0.98)
Peppara	*M. pinnata* (47.54)	--	*C. nucifera* (6.69) *Areca* sp. (10.21) Pollen type 21 (3.34) *M. peltata*(13.48) *M. pudica* (5.55) Pollen type 4 (7.51)	Pollen type 6 (2.61)
Kallara	Pollen type 22 (46.93)	*S. mahagoni* (34.29)	*C. nucifera* (4.22) *M. pinnata* (8.75) *Areca* sp. (9.46)	Pollen type 2 (0.64)
Tholicode	--	*M. pinnata* (34.02) *M. peltata*(18.63)	*C. nucifera* (13.57) *Areca* sp. (14.98) Pollen type 9 (7.04)	Pollen type 6 (1.98) Pollen type 4 (2.65) *M. pudica* (1.74)
Chennanpara	*D. regia* (63.88)	--	*P. pterocarpum* (13.5) *M. peltata* (14.01)	*M. pinnata* (1.75) *A. sessilis* (2.43) *M. pudica* (1.21)

were identified from Panacode. Least number of pollen types were in locations Perumpazhuthoor and Kulathoor which was four each.

C. nucifera (77.07 to 63.37 per cent) was distributed as predominant pollen type in locations Panacode and Melvettoor. *M. pinnata* (59.01 to 47.54 per cent) was present as predominantly found samples from Aruvippuram and Peppara whereas pollen of *M. peltata* (66.44 to 46.94 per cent) occurred predominantly in locations Poovar and Machel. *D. regia* (63.88 per cent) was predominant in Chennanpara while P. type 22 (46.93 per cent) occurred dominantly in Kallara, P. type 3 (46.96 per cent) in Panayamuttom and P. type 2 (60.15 per cent) in Perumpazhuthoor.

Secondary pollen types recorded included *C. nucifera*, *A. sessilis*, *M. pinnata*, *A. spinosus*, *S. mahagoni*, *M. peltata*, P. type 1 and P. type 5. *C. nucifera* was recorded from Amboori (18.89 per cent), Perumpazhuthoor (17.46 per cent), Kulathoor (44.9 per cent) and Powdikonam (37.89 per cent) whereas *A. sessilis* was recorded from Amboori (32.79 per cent). *M. pinnata* was recorded secondary pollen from locations Poovar (20.88 per cent), Perukavu (25.53 per cent) and Tholicode (34.02 per cent) while *M. peltata* was observed from Tholicode (18.63 per cent). *A. spinosus* was recorded as a secondary pollen in Panayamuttom (18.31 per cent) while *S. mahagoni* was recorded from Kallara (34.29 per cent) and Powdikonam (39.8 per cent). P. type 1 (43.15 to 30.04 per cent) was recorded as secondary pollen type from Kilimanoor and Kulathoor, P. type 5 (19.28 per cent) from Perumpazhuthoor and P. type 2 (17.19 per cent) from Melvettoor.

Important minor pollen recorded included *Areca* sp. (14.98 to 7.3 per cent), *M. pudica* (13.14 to 4.94 per cent), *M. pinnata* (12.87 to 3.4 per cent), *S. mahagoni* (4.92 per cent), *M. peltata* (14.01 to 3.05 per cent), *P. pterocarpum* (13.5 to 6.63 per cent), *P. guajava* (5.25 per cent), M. diplotricha (3.62 per cent), *C. nucifera* (14.89 to 4.22 per cent), *A. sessilis* (3.05 per cent), *Glochidion* sp. 2 (12.21 per cent), *A. conyzoides* (8.04 per cent), Myrtaceae sp. 1 (6.25 per cent), P. type 7 (4.26 per cent), P. type 20 (8.53 per cent), P. type 11 (7.5 per cent), P. type 1 (4.44 per cent), P. type 2 (12.1 to 10.58 per cent), P. type 3 (4.84 per cent), P. type 14 (4.14 per

cent), P. type 21 (3.34 per cent), P. type 4 (7.51 per cent) and P. type 9 (7.04 per cent).

Predominant pollen types were not observed in locations Kilimanoor, Amboori, Kulathoor, Powdikonam and Tholicode. Secondary pollen types were absent in Panacode, Aruvippuram, Machel, Melvettoor, Peppara and Chennanpara. Only one location, Perumpazhuthoor had no important minor pollen.

4.2.4 Distribution of pollen types in honey samples during dry season (January- May)

Distribution of pollen types in each honey samples in dry season is given in Table 7. The predominant pollen types were *C. nucifera*, *M. pinnata*, *M. pudica*, *Areca* sp., *C. pentandra*. Analysis revealed a total of 31 pollen types from 18 locations in which maximum was recorded in Aruvippuram and Thekkada which was 11 each. It is followed by Panayamuttom with ten pollen types, Peppara, Amboori and Panacode with nine pollen type each. Seven pollen types were recorded from Tholicode and Chennanpara and six each from Kilmanoor, Kulathoor and Kallara. Least number of pollen types were recorded from Kaviyode which was only 3.

C. nucifera (55.71 per cent) was found to be predominantly present in honey samples from Kulathoor meanwhile *M. pu*dica (51.97 per cent) was predominant in location Kilimanoor. Samples from Panacode had *M. pinnata* (66.95 per cent) as predomiant pollen type, *Areca* sp. (49.2 per cent) from Peppara and *C. pentandra* (49.32 per cent) from location Kallara. P. type 3 (92.93 per cent) was recorded as predominant from Kaviyode. Besides being predominant pollen type *C. nucifera*, *M. pinnata* and *Areca* sp. were also recorded as secondary and important minor pollen. *C. nucifera* was observed from Amboori (16.44 per cent) as secondary pollen but as important minor pollen from Panacode (3.5 per cent), Kaviyode (3.8 per cent), Thekkada (4.55 per cent), Panayamuttom (5.95 per cent), Peppara (10.28

Table 7. Distribution of pollen type classes in honey samples in dry season (January- May)

Locations	Pollen type classes and percentage			
	Predominant pollen	**Secondary pollen**	**Important minor pollen**	**Minor pollen**
Kilimanoor	*M. pudica* (51.97)	Pollen type 1 (34.14)	*M. pinnata* (4.61)	*M. diplotricha* (1.32) Pollen type 2 (1.97) *C. nucifera* (1.32)
Panacode	*M. pinnata* (66.95)	--	*M. pudica* (9.13) *A. spinosus* (3.91) Pollen type 11 (4.34) *M. peltata* (3.5) *C. nucifera* (3.5)	*P. pterocarpum* (2.61) *S. mahagoni* (1.3) *A. sessilis* (1.74)
Kaviyode	Pollen type 3 (92.93)	--	*C. nucifera* (3.8)	*M. pinnata* (1.6)
Amboori	--	*M. diplotricha* (27.39) *B. diffusa* (30.13) *C. nucifera* (16.44)	Pollen type 11 (15.1)	*A. sessilis* (2.94) Pollen type 5 (2.55) *M. pinnata* (2.64) Pollen type 2 (1.26) *D. regia* (1.37)
Kulathoor	*C. nucifera* (55.71)	*M. pinnata* (20) Pollen type 1 (17.14)	--	Pollen type 2 (2.85) *P. pterocarpum* (1.42) *Areca* sp. (1.43)
Aruvippuram	--	*M. pinnata* (40.59)	*M. peltata* (11.88) Pollen type 5 (8.91)	*C. nucifera* (2.97) *M. pudica* (2.97)

			Pollen type 3 (11.88) *Areca* sp. (3.96) Pollen type 2 (3.96) Pollen type 20 (6.93)	*P. pterocarpum* (1.98) *T. procumbens* (1.98)
Thekkada	--	Pollen type 2 (22.72) Pollen type 5 (34.09)	*M. pinnata* (11.36) *M. pudica* (4.55) Pollen type 10 (4.5) *C. nucifera* (4.55) *M. peltata* (4.55) Pollen type 12 (6.81)	*M. diplotricha* (2.27) *A. spinosus* (2.3) *A. conyzoides* (2.25)
Panayamuttom	--	Pollen type 5 (21.81) Pollen type 2 (34.37)	Pollen type 3 (3.12) *P. pterocarpum* (6.5) *A. spinosus* (7.1) *M. peltata* (6.5) *S. mahagoni* (5.4) *C. nucifera* (5.95)	*Croton* sp. (2.55) *T. procumbens* (2.9)
Peppara	*Areca* sp. (49.2)	--	*C. nucifera* (10.28) *A. spinosus* (6.95) Pollen type 2 (10.34) *P. edulis* (7.89) *N. indica* (3.45) Pollen type 4 (10.34)	Pollen type 3 (2.63) Pollen type 17 (2.45)

Kallara	*Ceiba pentandra* (49.32)	*S. mahagoni* (27.02)	*M. pinnata* (12.84)	Pollen type 1 (2.7) *M. pudica* (1.35) *C. nucifera* (1.5)
Tholicode	--	*Areca* sp. (24.21) *T. arjuna* (32.63) *A. sessilis* (28.42)	*C. nucifera* (10.52)	Pollen type 12 (1.05) *Trichosanthes* sp. (1.05) Pollen type 18 (2.11)
Chennanpara	--	*M. pinnata* (18.57) *A. sessilis* (18.57)	*P. pterocarpum* (11.43)	*A. conyzoides* (1.43) *C. nucifera* (1.43) *Areca* sp. (1.43) Pollen type 2 (1.43)

per cent) and Tholicode (10.52 per cent). *M. pinnata* meanwhile was recorded as secondary pollen type from Kulathoor (20 per cent), and Chennanpara (18.57 per cent) and as important minor pollen from Kilimanoor (4.61 per cent), Thekkada (11.36 per cent) and Kallara (12.84 per cent). *Areca* sp. was secondary pollen in Tholicode (21.41 per cent) while important minor in Aruvippuram (3.96 per cent).

Other secondary pollen types were *A. sessilis* (28.42 to 18.57 per cent) from Tholicode and Chennanpara, P. type 1 from Kilimanoor (34.14 per cent) and Kulathoor (17.14 per cent), P. type 2 (34.37 to 22.72 per cent) and P. type 5 (34.09 to 21.81 per cent) from Thekkada and Panayamuttom, *S. mahagoni* from Kallara (27.02 per cent), *M. diplotricha* (27.39 per cent) and *B. diffusa* (30.13 per cent) from Amboori and *T. arjuna* (32.63 per cent) from Tholicode.

Important minor plants included *M. pudica* (9.13 to 4.55 per cent), *A. spinosus* (7.1 to 3.91 per cent), *M. peltata* (11.88 to 3.5 per cent), *P. pterocarpum* (11.43 to 6.5 per cent), *S. mahagoni* (5.4 per cent), *P. edulis* (7.89 per cent), *N. indica* (3.45 per cent), P. type 3 (11.88 to 3.12 per cent), P. type 11 (15.1 to 4.34 per cent), P. type 2 (10.34 to 3.96 per cent), P. type 4, P. type 5, P. type 20, P. type 12, P. type 10 and P. type 9.

Predominant pollen types were not recorded in honey samples from locations Amboori, Aruvippuram, Thekkada, Panayamuttom, Tholicode and Chennanpara. No secondary pollen type was recorded from locations Panacode, Kaviyode and Peppara whereas important minor pollen types were absent in Kulathoor.

4.2.5 Distribution of pollen types in pollen loads during southwest monsoon season (June- September)

Pollen distribution of the season southwest monsoon (June- September) in the pollen loads is given in Table 8. A total of 31 pollen types were revealed in the analysis in which eight pollen types occurred in Panacode and Thekkada which was the maximum number of pollen type in the season. Least number (5) was observed from Aruvippuram and Melvettoor. Seven pollen types were recorded from

Table 8. Distribution of pollen type classes in pollen loads in southwest monsoon season (June- September)

Locations	**Pollen type classes and percentage**			
	Predominant pollen	**Secondary pollen**	**Important minor pollen**	**Minor pollen**
Kilimanoor	*M. pudica* (88.1)	--	Pollen type 7 (7.92)	*Areca* sp. (1.62) *C. nucifera* (1.38)
Panacode	*M. pinnata* (55.1)	--	Pollen type 16 (8.36) *M. peltata*(13.22) *A. sessilis* (6.69) *M. pudica* (7.86)	*C. nucifera* (1.99) Pollen type 3 (1.27) *P. pterocarpum* (1.51)
Kaviyode	*A. catechu* (94.94)	--		Poaceae sp. 1 (2.02) *P. edulis* (2.02) Pollen type 8 (1.01)
Amboori	--	*A. sessilis* (33.9) *M. peltata* (25.14)	*C. nucifera* (15.82) *T. procumbens* (14.69) *M. pudica* (7.34)	*Areca* sp. (0.84) *P. pterocarpum* (0.84)
Perumpazhuthoor	*M. pudica* (71.68)	*C. nucifera* (25.39)	--	*A. latronum* (0.84) *A. conyzoides* (0.73) *T. arjuna* (0.42)
Kulathoor	*C. nucifera* (61.33)	*M. pinnata* (29.23)	Pollen type 1 (8.11)	*Areca* sp. (1.03)
Poovar	*M. pudica* (57.99)	*C. nucifera* (32.83)	*M. pinnata* (5.97)	Pollen type 1 (2.13)
Aruvippuram	*C. nucifera* (87.57)	--	*A. conyzoides* (10.17)	*Areca* sp. (1.69)
Machel	*C. nucifera* (71.1)	*M. peltata* (19.11)	Pollen type 5 (3.11)	*M. pinnata* (1.33) Pollen type 8 (1.33) Pollen type 7 (1.77)

Thekkada	--	*M. pinnata* (20.57) *M. peltata*(32.74) *M. pudica* (16.52)	*Areca* sp. (12.46) *A. sessilis* (5.44) *C. nucifera* (4.05)	Pollen type 9 (1.28) Pollen type 5 (2.37)
Powdikonam	*M. pudica* (85.9)	--	*C. nucifera* (4.32) *M. diplotricha* (5.29)	*A. conyzoides* (1.82) Pollen type 9 (1.03)
Panayamuttom	Arecaceae sp. 1 (66.44)	*A. conyzoides* (19.62)	*C. nucifera* (9.92)	Pollen type 4 (2.79)
Melvettoor	*M. pinnata* (91.35)	--	*C. nucifera* (5.1)	*Glochidion* sp. 1 (0.94)
Perukavu	Pollen type 4 (59.42)	*A. mangium* (21.26) *M. peltata*(17.58)	--	*M. pudica* (0.77)
Peppara	*M. pinnata* (60.4)	--	*Areca* sp. (9.8) Pollen type 4 (9.7) *C. nucifera* (4.7)	Pollen type 3 (2) Pollen type 15 (1.2)
Kallara	--	*M. pudica* (42.73) *M. pinnata* (27.84)	*S. mahagoni* (7.86) *M. diplotricha* (10.83) *A. conyzoides* (6.27)	*A. spinosus* (1.92) *C. nucifera* (1.58)
Tholicode	*M. pudica* (82.28)	--	*C. nucifera* (9.81) Poaceae sp.1 (14.5)	*Areca* sp. (2.05) C. *pulcherrima* (1.02) *S. nodiflora* (1.47)
Chennanpara	*M. pudica* (73.26)	--	*P. pterocarpum* (10.07) *C. nucifera* (4.92) *M. peltata* (6.71)	*A. mangium* (2.04) Pollen type 11 (1.2)

Amboori and Kallara, whereas Machel, Peppara, Tholicode and Chennanpara had six pollen types each. Perumpazhuthoor and Powdikonam had 5 pollen types each and four pollen types were observed in samples from Kilimanoor, Kaviyode, Kulathoor, Poovar, Panayamuttom and Perukavu.

The pollen type distribution revealed that *M. pudica* (88.1 to 57.99 per cent) was recorded as predominant type from maximum number of locations, Kilimanoor, Perumpazhuthoor, Poovar, Powdikonam, Tholicode and Chennanpara. *C. nucifera* (87.57 to 61.33 per cent) was predominant in three loactions, namely, Kulathoor, Aruvippuram and Machel. *M. pinnata* (91.35 to 55.1 per cent) was recorded to be dominant in Panacode, Melvettoor and Peppara. *A. mangium* (94.94 per cent), Arecaceae sp. 1 (66.44 per cent), P. type 4 (59.42 per cent) was found to be predominant in one location each, which were Kaviyode, Panayamuttom and Perukavu respectively.

C. nucifera was also reported as secondary pollen type from Perumpazhuthoor (25.39 per cent) and Poovar (32.83 per cent) and important minor pollen from Amboori (15.82 per cent), Thekkada (4.05 per cent), Powdikonam (4.32 per cent), Panayamuttom (9.92 per cent), Melvettoor (5.1 per cent), Peppara (4.7 per cent), Tholicode (9.81 per cent) and Chennanpara (4.92 per cent). Whereas *M. pudica* was recorded to be secondary pollen type from Thekkada (16.52 per cent) and Kallara (42.73 per cent) and as important minor pollen from Panacode (7.86 per cent) and Amboori (7.34 per cent). From three locations, Kallara (27.84 per cent), Thekkada (20.57 per cent) and Kulathoor (29.23 per cent) *M. pinnata* was recorded as secondary pollen type while from one location, Poovar (5.97 per cent) it was recorded as important minor pollen. *M. peltata* was recorded as secondary pollen from Amboori (25.14 per cent), Machel (19.11 per cent), Thekkada (32.74 per cent) and Perukavu (17.58 per cent) while it was important minor pollen in Panacode (13.22 per cent) and Chennanpara (6.71 per cent). *A. sessilis* was found to be secondary pollen in Amboori (33.9 per cent) whereas an important minor pollen from Panacode (6.69 per cent) and Thekkada (5.44 per cent). Secondary pollen *A. conyzoides* observed in Panayamuttom (19.62 per cent) was also observed

as important minor in Kallara (6.27 per cent) and Aruvippuram (10.17 per cent). *A. mangium* (21.26 per cent) was also a secondary pollen during the season in Perukavu.

Important minor pollen included *T. procumbens* (14.69 per cent), *Areca* sp. (9.8 to 12.46 per cent), *M. diplotricha* (10.83 to 5.29 per cent), *S. mahagoni* (7.86 per cent), Poaceae sp. 1 (14.5 per cent), *P. pterocarpum* (10.07 per cent), P. type 1, P. type 4, P. type 5, P. type 7, P. type 16. *T. procumbens* was recorded from Amboori, *Areca* sp. from Thekkada and Peppara, *M. diplotricha* from Powdikonam and Kallara, *S. mahagoni* from Kallara, and *P. pterocarpum* from Chennanapara. Three locations *viz*., Amboori, Thekkada and Kallara had no predominant pollen type while Kilimanoor, Panacode, Kaviyode, Aruvippuram, Powdikonam, Melvettoor, Peppara, Tholicode and Chennanpara recorded no secondary pollen type. Important minor pollen type were not observed from Kaviyode, Perumpazhuthoor and Perukavu.

4.3 FREQUENCY OF OCCURRENCE OF POLLEN TYPES

A total of 115 pollen types were recorded from the samples of honey and pollen samples.

4.3.1 Frequency of occurrence of pollen types in honey samples

Frequency of occurrence of pollen types in honey samples when calculated showed that *C. nucifera* and *M. pinnata* were the very frequent pollen types (Table 9). Out of 12 locations from where honey samples were collected in dry season *C. nucifera* occurred in all 12 locations and out of 9 honey samples in northeast monsoon season it occurred in 6 locations. *M. pinnata* occurred in 9 samples in dry season while in 6 locations in northeast monsoon season.

Five pollen types were recorded as frequent in northeast monsoon season, namely *M. pudica, M. diplotricha, M. peltata, Areca* sp. and *T. procumbens* while 11 pollen were found to be frequent during dry season. It included *M. pudica, M.*

Table 9. Frequency of occurrence of pollen types in honey samples

Seasons	Very frequent	Frequent	Infrequent	Rare
Northeast monsoon season	*C. nucifera* *M. pinnata*	*M. pudica* *M. diplotricha* *M. peltata* *Areca sp.* *T. procumbens*	*P. foetida* *M. micrantha*	*P. pterocarpum* *C. grandis* *C. pulcherrima* Poaceae sp. 2 *J. procumbens* *Eucalyptus sp.* Asteraceae sp. 1 Pollen type 1 Pollen type 5 Pollen type 19 Pollen type 9
Dry season	*C. nucifera* *M. pinnata* Pollen type 2	*M. pudica* *M. diplotricha* *A. spinosus* *M. peltata* *P. pterocarpum* *S. mahagoni* *A. sessilis* *Areca sp.* Pollen type 1 Pollen type 4 Pollen type 5	*T. procumbens* *A. conyzoides* Pollen type 11	*B. diffusa* *D. regia* *Croton sp.* *P. edulis* *N. indica* *C. pentandra* *T. arjuna* *C. inophyllum* *Trichosanthes sp.* Pollen type 20 Pollen type 10 Pollen type 12 Pollen type 4 Pollen type 17 Pollen type 18

diplotricha, *A. spinosus*, *M. peltata*, *P. pterocarpum*, *S. mahagoni*, *A. sessilis* and *Areca* sp.

Infrequent pollen types during northeast monsoon season included *P. foetida* and *M. micrantha* which occurred only in 2 locations. While there were 3 pollen types, *T. procumbens*, *A. conyzoides* and P. type 11 during dry season.

Rare pollen types (occurring only in one location) during northeast monsoon season were 11 in number which includes *P. pterocarpum*, *Coccinia grandis*, *C. pulcherrima*, *J. flava*, *Eucalyptus* sp., Asteraceae sp. 1, Poaceae sp. 2, P. type 1, 5, 19, 12. While rare pollen types in dry season numbered 14, namely, *B. diffusa*, *D. regia*, *Croton* sp., *P. edulis*, *N. indica*, *C. pentandra*, *T. arjuna*, *C. inophyllum*, P. type 4, 10, 12, 17, 20, 25.

4.3.2 Frequency of occurrence of pollen types in pollen loads

Frequency of occurrence of pollen types showed that *C. nucifera* and *M. pudica* were the most frequent pollen type throughtout the year in pollen loads (Table 10). During northeast monsoon season and dry season, *M. pinnata* was also very frequent pollen and *M. peltata* was observed as very frequent only in dry season. *C. nucifera* occurred in 15 locations out of 17 during northeast monsoon, 15 out of 16 during dry and 16 out of 18 in southwest monsoon season. *M. pudica* was present in 15 locations during northeast monsoon season, 8 locations during dry and 11 locations during southwest monsoon season.

In northeast monsoon and southwest monsoon seasons, *M. peltata* was observed to be frequent pollen type (occurring in number of locations 4-10) in samples, while *Areca* sp. was frequently recorded during all seasons in pollen samples. *P. pterocarpum* was frequently present in pollen loads during northeast monsoon and dry whereas in southwest monsoon season it was recorded under infrequent type. *M. pinnata* was recorded as frequent during southwest monsoon season. Other frequent pollen during northeast monsoon season were *T.*

Table 10. Frequency of occurrence of pollen types in pollen loads

Seasons	Very frequent	Frequent	Infrequent	Rare
Northeast monsoon season	*C. nucifera* *M. pudica* *M. pinnata*	*M. peltata* *Areca* sp. *T. procumbens* *M. diplotricha* *M. micrantha* *P. pterocarpum* *B. flabellifer* *A. conyzoides* Pollen type 6	*A. viridis* *P. foetida* *T. arjuna* *A. sessilis* *P. araca* Pollen type 1 Pollen type 9 Pollen type 10 Pollen type 11	*D. regia* *S. parasitica* *C. citrinus* *A. latronum* *Eucalyptus sp.* *J. procumbens* *J. gendurusso* *D. falcata* *A. hybridus* *E. glomeratus* *M. malabathricum* Arecaceae sp. 1 Pollen type 2 Pollen type 13
Dry season	*M. pudica* *M. pinnata* *C. nucifera* *M. peltata*	*S. mahagoni* *P. pterocarpum* *A. sessilis* *Areca* sp. Pollen type 1 Pollen type 2 Pollen type 4 Pollen type 6	*P. guajava* *A. conyzoides* *B. flabellifer* *Glochidion* sp. 2 *A. spinosus* Pollen type 3 Pollen type 5 Pollen type 9 Pollen type 11	*T. arjuna* *M. diplotricha* *D. falcata* *D. regia* *C. inophyllum* *I. coccinea* Myrtaceae sp. 1 Poaceae sp.1 Pollen type 7 Pollen type 14 Pollen type 20 Pollen type 21 Pollen type 22

Southwest monsoon season	*M. pudica* *C. nucifera*	*Areca sp.* *M. peltata* *M. pinnata*. *A. conyzoides*	*A. sessilis* *P. pterocarpum* *A. mangium* *M. diplotricha* Poaceae sp.1 Pollen type 1 Pollen type 4 Pollen type 5 Pollen type 7 Pollen type 8 Pollen type 9	*P. edulis* *T. procumbens* *A. catechu* *T. arjuna* *S. mahagoni* *A. spinosus* *C. pulcherrima* *Chromolaena sp.* *Glochidion* sp. 1 Arecaceae sp. 1 Pollen type 3 Pollen type 8 Pollen type 11 Pollen type 15 Pollen type 16

procumbens, M. diplotricha, M. micrantha, B. flabellifer, A. conyzoides and P. type 6.

Pollen types that were frequent only during dry season were *S. mahagoni*, *A. sessilis*, P. type 1, 2, 4 and 6 while in southwest monsoon season it were *A. conyzoides*. The infrequent pollen types (occurring in 2-3 locations) during northeast monsoon season were *A.viridis, P. foetida*, *T. arjuna, A. sessilis, P. araca*, P. type 1, 9, 10 and 11. *P. guajava, A. conyzoides, B. flabellifer, Glochidion* sp. 2, *A. spinosus*, P. type 3, 5, 9 and 11 were recorded during dry season. Eleven pollen types were infrequent during southwest monsoon season, namely, *A. sessilis, P. pterocarpum, A. mangium, M. diplotricha*, Poaceae sp. 1, P. type 1, 4, 5, 7, 8 and 9.

Fourteen pollen types occurred as rare type (occurring only in one location) during northeast monsoon season which were *D. regia*, *S. parasitica*, *C. citrinus, A. latronum*, *Eucalyptus* sp., *J. procumbens, J. gendarussa, D. falcata, A. hybridus*, Arecaceae sp. 1, P. type 2, 13, 14 and 18. The pollen that were rare during dry season were *T. arjuna*, *M. diplotricha, D. falcata*, *D. regia*, *C. inophyllum, Ixora coccinea*, Myrtaceae sp.1, Poaceae sp.1, P. type 7, 20, 21, 22 and 24. *P. edulis, T. procumbens, A. latronum, T. arjuna, S. mahagoni, A. spinosus, C. pulcherrima, Chromolaena* sp., *Glochidion* sp.1, Arecaceae sp. 1, P. type 3, 8, 11, 15, 16 and 17 were rare during southwest monsoon season.

4.4 SEASONAL VARIATION

The seasonal variation in pollen types is represented in the Table 11. There was a considerable difference in the pollen types obtained from eighteen locations during the three seasons. Maximum number of pollen types was recorded during dry season (78), followed by northeast monsoon season (67) and southwest monsoon season (62). Pollen types which was recorded during all the three seasons were *C. nucifera, M. pudica, M. pinnata, M. diplotricha, P. pterocarpum, D. regia, A. sessilis, M. peltata, Areca* sp., *B. flabellifer, A. latronum, A. conyzoides, T. arjuna, C. pulcherrima, C. pentandra, P. edulis, M. charantia, T. procumbens, A. spinosus, V. negundo, C. arborea, E. glomeratus, Manihot* sp., *Syzygium cumini*,

Table 11. Seasonal variation in pollen types among the seasons

Sl. No.	Northeast monsoon	Dry	Southwest monsoon
1	*C. nucifera*	*C. nucifera*	*C. nucifera*
2	*M. pudica*	*M. pudica*	*M. pudica*
3	*M. pinnata*	*M. pinnata*	*M. pinnata*
4	*M. diplotricha*	*M. diplotricha*	*M. diplotricha*
5	*A. sessilis*	*A. sessilis*	*A. sessilis*
6	*P. pterocarpum*	*P. pterocarpum*	*P. pterocarpum*
7	*M. peltata*	*M. peltata*	*M. peltata*
8	*Areca* sp.	*Areca* sp.	*Areca* sp.
9	*T. procumbens*	*T. procumbens*	*P. edulis*
10	*M. micrantha*	*S. mahagoni*	*A. mangium*
11	*A. viridis*	*M. micrantha*	*A. catechu*
12	*D. regia*	*B. diffusa*	*A. latronum*
13	*B. flabellifer*	*A. spinosus*	*T. arjuna*
14	*S. parasitica*	*D. regia*	*A. conyzoides*
15	*A. conyzoides*	*A. latronum*	*B. pilosa*
16	*P. foetida*	*A. mangium*	*A. spinosus*
17	*A. latronum*	*A. conyzoides*	*Glochidion* sp.1
18	*A. catechu*	*P. edulis*	*S. mahagoni*
19	*Eucalyptus* sp.	*C. pentandra*	*C. pulcherrima*
20	*J. procumbens*	*T. arjuna*	*C. pentandra*
21	*J. gendurussa*	*B. flabellifer*	*T. gamblei*
22	*D. falcata*	*C. citrinus*	*B. flabellifer*
23	*T. arjuna*	*P. araca*	*C. thomsonae*
24	*P. araca*	*P. guajava*	*M. charantia*
25	*A. hybridus*	*I. coccinea*	*P. guajava*
26	*C. pulcherrima*	*Croton* sp.	*E. heterophylla*
27	*Coccinia grandis*	*Glochidion* sp.2	*T. procumbens*
28	*E. heterophylla*	*N. indica*	*D. regia*
29	*E. pulcherrima*	*Eucalyptus* sp.	*P. foetida*
30	*P. edulis*	*Helixanthera* sp.	*Syzygium cumini*
31	*C. citrinus*	*M. charantia*	*S. nodiflora*
32	*C. pentandra*	*Coccinia grandis*	*L. siceraria*
33	*O. gratissimum*	*Justicia adhatoda*	*Jatropha* sp.
34	*M. charantia*	*Macrosolen* sp.	*A. viridis*

35	*A. spinosus*	*Manihot* sp.	*P. urinaria*
36	*C. bonduc*	*M. citrifolia*	*E. guineensis*
37	*A. squamosa*	*C. pulcherrima*	*A. squamosa*
38	*V. negundo*	*T. potulacastrum*	*V. negundo*
39	*C. arborea*	*Syzygium cumini*	*T. tricuspidata*
40	*M. malabathricum*	*Syzygium* sp.	*Trichosanthes* sp.
41	*Citrus* sp.	*S. malabaricum*	*C. arborea*
42	*Manihot* sp.	*L. siceraria*	*Manihot* sp.
43	*Syzygium* sp.	*C. inophyllum*	*E. glomeratus*
44	*E. glomeratus*	*B. hispida*	*J. betonica*
45	*J. pentantha*	*E. glomeratus*	*S. parasitica*
46	*M. hirtus*	*Lilium* sp.	Caryophyllaceae sp. 2
47	*M. vitifolia*	*Chromolaena* sp.	Poaceae sp. 1
48	*Macrosolen* sp.	*S. nodiflora*	Malvaceae sp. 1
49	*Spermacoce* sp. 1	*A. muricata*	Arecaceae sp. 1
50	Poaceae sp. 1	*T. diverticata*	P type 1
51	Poaceae sp. 2	*T. gamblei*	P type 2
52	Arecaceae sp. 1	*V. negundo*	P type 3
53	Arecaceae sp. 2	*C. arborea*	P type 4
54	Asteraceae sp. 1	*Trichosanthes* sp.	P type 5
55	Rutaceae sp. 1	*H. malabarica*	P type 6
56	Caryophyllaceae sp. 1	*Spermacoce* sp. 2	P type 7
57	P type 1	Poaceae sp. 1	P type 8
58	P type 2	Arecaceae sp. 1	P type 9
59	P type 3	Myrtaceae sp. 1	P type 11
60	P type 5	Caryophyllaceae sp. 1	P type 15
61	P type 6	P type 1	P type 16
62	P type 9	P type 2	P type 22
63	P type 8	P type 3	
64	P type 10	P type 4	
65	P type 11	P type 5	
66	P type 13	P type 6	
67	P type 19	P type 7	
68		P type 8	
69		P type 9	
70		P type 10	

71		P type 11	
72		P type 12	
73		P type 14	
74		P type 17	
75		P type 18	
76		P type 20	
77		P type 21	
78		P type 22	

Poaceae sp. 1, Arecaceae sp. 1, P. type 1, P. type 2, P. type 3, P. type 5, P. type 6, P. type 8, P. type 9 and P. type 11.

P. araca, *M. micrantha*, *Eucalyptus* sp., *Coccinia grandis*, *C. citrinus*, *Macrosolen* sp., Caryophyllaceae sp. 1 and P. type 10 were recorded in northeast monsoon and dry season. The pollen types recorded during both dry season and southwest monsoon season included *S. mahagoni*, *A. mangium*, *P. guajava*, *L. siceraria*, *S. nodiflora*, *T. gamblei*, *Trichosanthes* sp., P. type 7, P. type 9 and P. type 22. *A. viridis*, *P. foetida*, *A. catechu*, *E. heterophylla*, *S. parasitica* and *A. squamosa* were recorded both in northeast monsoon and southwest monsoon seasons.

J. procumbens, *J. gendarussa*, *D. falcata*, *A. hybridus*, *E. pulcherrima*, *O. gratissimum*, *C. bonduc*, *M. malabathricum*, *J. pentantha*, *M. hirtus*, *M. vitifolia*, *Citrus* sp., *Spermacoce* sp. 1, Poaceae sp. 2, Arecaceae sp. 2, Asteraceae sp. 1, Rutaceae sp. 1, P. type 13 and P. type 19 were recorded exclusively in northeast monsoon season. *P. guajava*, *B. diffusa*, *I. coccinea*, *Croton* sp., *N. indica*, *Helixanthera* sp., *J. adhatoda*, *M. citrifolia*, *T. potulacastrum*, *S. malabaricum*, *C. inophyllum*, *Lilium* sp., *A. muricata*, *B. hispida*, *Chromolaena* sp., *H. malabarica*, *T. diverticata*, *Spermacoce* sp. 2, *Glochidion* sp. 2, Myrtaceae sp. 1, P. type 12, P. type 14, P. type 17, P. type 18, P. type 20 and P. type 21 were recorded only during dry season. The pollen types which were observed only during southwest monsoon season were *B. pilosa*, *C. thomsonae*, *Jatropha* sp., *P. urinaria*, *E. guineensis*, *J. betonica*, *T. tricuspidata*, *Glochidion* sp. 1, Caryophyllaceae sp. 2, Malvaceae sp. 1, P. type 15 and P. type 16.

4.5 FAMILY AND PLANT GROUP DISTRIBUTION OF POLLEN TYPES

4.5.1 Botanical family representation of pollen types

The family wise distribution of pollen types are given in Table 12. Pollen types were recognized into a total of 31 families. The maximum number of pollen were observed in family Fabaceae (8) followed by Myrtaceae and Asteraceae

Table 12. Botanical family representation in identified pollen types

Sl. No.	Family name	No. of species	Scientific name
1	Arecaceae	6	*Cocos nucifera* *Borassus flabellifer* *Areca* sp. *Elaeis guineensis* Arecaceae sp. 1 Arecaceae sp. 2
2	Asteraceae/ Compositae	7	*Tridax procumbens* *Mikania micrantha* *Ageratum conyzoides* *Bidens pilosa* *Chromolaena* sp. *Synedrella nodiflora* Asteraceae sp. 1
3	Amaranthaceae	4	*Alternanthera sessilis* *Amaranthus spinosus* *Amaranthus viridis* *Amaranthus hybridus*
4	Fabaceae	8	*Mimosa pudica* *Mimosa diplotricha* *Milletia pinnata* *Peltophorum pterocarpum* *Acacia mangium* *Acacia latronum* *Acacia catehu* *Delonix regia*
5	Euphorbiaceae	6	*Macaranga peltata* *Croton* sp. *Jatropha* sp. *Euphorbia heterophylla* *Euphorbia pulcherrima* *Manihot* sp.

6	Caesalpinaceae	2	*Caesalpinia pulcherrima* *Caesalpinia bonduc*
7	Nyctaginaceae	1	*Boerrhavia diffusa*
8	Combretaceae	1	*Terminalia arjuna*
9	Loranthaceae	4	*Scurrula parasitica* *Dendrophthoe falcata* *Macrosolen* sp. *Helixanthera sp.*
10	Myrtaceae	7	*Psidium guajava* *Psidium araca* *Eucalyptus* sp. *Syzygium cumini* *Syzygium* sp. *Callistemon citrinus* Myrtaceae sp. 1
11	Malvaceae	2	*Ceiba pentandra* Malvaceae sp. 1
12	Passifloraceae	2	*Passiflora edulis* *Passiflora foetida*
13	Phyllanthaceae	3	*Glochidion* sp.1 *Glochidion* sp. 2 *Phyllanthus urinaria*
14	Meliaceae	1	*Sweitenia mahagoni*
15	Acanthaceae	4	*Justicia procumbens* *Justicia gendarussa* *Justicia adhatoda* *Justicia betonica*
16	Menyanthaceae	1	*Nymphoides indica*
17	Cucurbitaceae	6	*Coccinia grandis* *Momordica charantia* *Lagenaria siceraria* *Benincasa hispida* *Trichosanthes tricupidata*

			Trichosanthes sp.
18	Rubiaceae	5	*Ixora coccinea* *M. citrifolia* *M. hirtus* *Spermacoce* sp. 1 *Spermacoce* sp. 2
19	Rutaceae	2	*Citrus* sp. Rutaceae sp. 1
20	Poaceae	2	Poaceae sp. 1 Poaceae sp. 2
21	Lamiaceae	3	*Ocimum gratissimum* *Vitex negundo* *Clerodendron thomsonae*
22	Convolvulaceae	4	*Evolvulus* sp. *M. vitifolia* *H. malabarica* *J. pentantha*
23	Liliaceae	1	*Lilium* sp.
24	Pedaliaceae	1	*Sesamum malabaricum*
25	Lecythidaceae	1	*Careya arborea*
26	Annonaceae	2	*Annona muricata* *Annona squamosa*
27	Calophyllaceae	1	*Calophyllum inophyllum*
28	Apocynaceae	2	*Tabernaemontana gamblei* *Tabernaemontana diverticata*
29	Aizoaceae	1	*Trianthema potulacastrum*
30	Caryophyllaceae	2	Caryophyllaceae sp. 1 Caryophyllaceae sp. 2
31	Melastomaceae	1	*Melastoma malabathricum*

having 7 pollen types each. It was followed by families Arecaeae, Euphorbiaceae and Cucurbitaceae which had six pollen types each. Least number of pollen was contributed by families Nyctaginaceae, Combretaceae, Meliaceae, Menyathaceae, Liliaceae, Pedaliaceae, Lecythidaceae, Callophyllaceae, Melastomaceae and Aizoaceae which had one pollen type. Two pollen types were recorded from families Passifloraceae, Malvaceae, Rutaceae, Poaceae, Caryophyllaceae, Annonaceae, Caesalpiniaceae and Apocynaceae. Family Lamiaceae and Phyllanthaceae had three pollen types whereas Amaranthaceae, Loranthaceae, Acanthaceae and Convolvulaceae had four pollen types each. Five pollen types were recorded only from Rubiaceae family.

4.5.2 Relative abundance of plant groups in identified pollen types

When the relative abundance according to plant type was calculated in the 83 plant species identified atleast upto species level, the highest abundance was observed for medicinal plants, followed by weeds, fruits or plantation crops, trees, ornamentals, vegetables and then oilseeds (Table 13). The medicinal plants were 27 in number and weeds were 16 in number. It was followed by fruits and plantation crops which was 14 in number. Ten pollen types were from tree species, 7 vegetables species, 8 ornamental species and 1 oilseed species were also present in the pollen types.

4.6 POLLEN METABARCODING

The pollen samples from three seasons were subjected to metabarcoding using primers rbcLa, rbcLb, psb, tnrH, ITS4, ITS5, matK1 and mat K3. The sequence reads that could be characterised into species with high hit result were *Amaranthus palmeri, A. tuberculatus, A. caudatus, A. spinosus, A. hybridus, A. hypochondriacus, A. viridis, Malvales* sp., *Benicasa hispida* and *Lagenaria siceraria*. Other species with slightly less predictable barcode matches from the sequencing were *Muntingia calabura, Citrullus rehmii, C. mucosospermus, C. colocynthis C. lanatus, Reevesia thyrsoides, Myriophyllum spicatum, Vatica bella, Scaphopetalum blackii* and *Shorea roxbhurgii.* Species that were comparatively

Table 13. Composition and relative abundance of plant groups among identified pollen types

Sl. No.	Plant type	No. of pollen types	% Abundance
1	Trees	10	12.05
2	Fruits and plantation	14	16.87
3	Medicinal and aromatics	27	32.53
4	Vegetables	7	8.43
5	Ornamentals	8	9.64
6	Weeds	16	19.28
7	Oilseeds	1	1.2

least predictable included *Dipentodon sinicus, Alcea rosea, Barbeya oleoides, S. peltata, Microcos coriacea, Althaea officinalis* and *Vateria copallifera.* All other species that are recorded in metabarcoding are very minor matches, hence cannot be considered effective.

The important sources of bees like *C. nucifera, M. pudica* and *M. pinnata* are not in the results which might be due to the fact that DNA isolated was degraded very fastly and the process was not repeated due to high costs of metabarcoding. Also the technique requires high skills and since it was attempted for the first time human-made errors might have happened leading to an incomplete result.

The entire list of pollen types identified through metabarcoding is given in Table 14.

4.7 POLLEN DENSITY

The total pollen density of honey samples per mL collected during two seasons, namely northeast monsoon and dry are represented below in Table 15. The honey samples during southwest monsoon season was not collected since the amount of honey in colonies were deficient for the colony purposes.

The honey samples were obtained from 9 locations during the northeast monsoon season. The TNP/ mL obtained for the honey samples ranged from 44,000 (Group II) to 2, 08,000 (Group III). Samples from seven out of nine locations were classified into Group III (1, 01,500- 2, 08,000 TNP/mL) whereas two locations were classified as Group II (44,000-83,000 TNP/mL). The honey samples obtained during dry season numbered 13. The TNP/mL ranged from 27,000 (Group II) to 2, 94,000 (Group III). Out of the 13 locations, seven locations had honey samples categorised into Group II (27,000- 92,000 TNP/mL) and remaining Six locations had honey categorised into Group III (1, 22,000- 2, 94,000 TNP/mL). None of the samples were classified as Group I, IV or V.

When the absolute pollen count per ml of honey was considered the highest density was at the location Amboori (2, 08, 000 grains/mL) followed by

Table 14. Plant species of pot pollen samples based on metabarcoding

Sl. No.	Species name	Bit score	E- value
1	*Amaranthus palmeri*	542	6e-150
2	*Amaranthus tuberculatus*	532	3e-147
3	*Amaranthus hybridus*	521	7e-144
4	*Amaranthus caudatus*	536	3e-148
5	*Amaranthus hypochondriacus*	536	3e-148
6	*Amaranthus spinosus*	531	1e-146
7	*Amaranthus viridis*	518	1e-142
8	*Malvales sp.*	459	6e-125
9	*Benincasa hispida*	459	1e-124
10	*Lagenaria siceraria*	451	2e-122
11	*Muntingia calabura*	326	6e-85
12	*Citrullus rehmii*	313	4e-81
13	*Citrullus colocynthis*	313	4e-81
14	*Citrullus mucosospermus*	307	2e-79
15	*Citrullus lanatus subsp. vulgaris*	307	2e-79
16	*Reevesia thyrsoidea*	228	2e-55
17	*Myriophyllum spicatum*	224	2e-54
18	*Vatica bella*	222	9e-54
19	*Scaphopetalum blackii*	211	2e-50
20	*Shorea roxburghii*	211	2e-50
21	*Dipentodon sinicus*	206	7e-49
22	*Alcea rosea*	204	3e-48
23	*Barbeya oleoides*	200	3e-47
24	*Shorea peltata*	200	4e-47
25	*Microcos coriacea*	196	4e-46
26	*Althaea officinalis*	198	1e-46
27	*Vateria copallifera*	176	6e-40

28	*Shorea longisperma*	167	3e-37
29	*Shorea longiflora*	167	3e-37
30	*Shorea faguetioides*	167	3e-37
31	*Shorea faguetiana*	167	3e-37
32	*Vatica oblongifolia*	161	2e-35
33	*Escallonia pulverulenta*	137	3e-28
34	*Escallonia illinita*	134	4e-27
35	*Escallonia revoluta*	134	4e-27
36	*Hibiscus meyeri*	132	1e-26
37	*Corchorus olitorius*	132	1e-26
38	*Prunus serotina*	132	1e-26
39	*Escallonia tucumanensis*	128	2e-25
40	*Escallonia myrtilloides*	128	2e-25
41	*Escallonia millegrana*	128	2e-25
42	*Escallonia discolor*	128	2e-25
43	*Passiflora arbelaezii*	124	2e-24
44	*Escallonia alpina*	121	3e-23
45	*Tapiscia sinensis*	117	3e-22
46	*Prunus undulata*	111	2e-20
47	*Prunus avium*	104	3e-18
48	*Prunus campanulata*	104	3e-18

Table 15. Total pollen count of honey samples

Location	Northeast monsoon	Dry
Kilimanoor	1,21,100	42,000
Poovar	1,57,000	2,09,000
Panacode	1,52,000	2,94,000
Aruvippuram	44,000	27,000
Amboori	2,08,000	60,000
Peppara	83,000	1,22,000
Chennanpara	1,83,000	2,52,000
Powdikonam	1,37,000	--
Tholicode	1,01,500	92,000
Kaviyode	--	1,52,000
Panayamuttom	--	61,000
Vembayam	--	84,000
Kulathoor	--	2,45,000
Kallara	--	49,000

Chennanpara and Poovar. The least density was counted in honey from location at Aruvippuram (44,000/mL). The honey samples collected during dry season accounted for 13 locations. The highest absolute pollen count was found in location Panacode (2, 94,000 grains/mL) followed by Chennanpara and Kaviyode. The least density was found in the location Aruvippuram (27,000 grains/mL).

There was also variations in pollen density in the same location between seasons. The pollen density of locations Panacode, Peppara and Chennanpara showed an increase during dry season when compared to northeast monsoon season. While locations Kilimanoor, Poovar, Aruvippuram, Amboori and Tholicode is marked by a considerable decline during the dry season. In both the seasons least density was found from location Aruvippuram.

When the pollen density of samples from midlands are considered in both the seasons, northeast monsoon season had more pollen density while there was a marked decline during dry season. Although in uplands, the pollen density was comparatively lower in northeast monsoon season than in dry season. The pollen density between midland and upland within the north-east monsoon season were not significantly varying. Whereas in dry season the pollen density was remarkably higher in uplands when compared to most of the midlands.

4.8 FORAGING ACTIVITY OF STINGLESS BEE

Foraging activity of stingless bee, *Tetragonula travancorica* was studied during the period November 2018 to August 2019 in three seasons, namely northeast monsoon, dry and southwest monsoon season.

4.8.1 Activity of outgoing foragers

4.8.1.1 During northeast monsoon (November to December)

The activity of outgoing foragers continued throughout the day in all months of the season (Table 16). The lowest activity was observed during 0600 to 0700 h with a mean of 2.14 bees/5 min/h. A peak of activity was observed during 0800 to

Table 16. Foraging activity of outgoing foragers in northeast monsoon season

Months↓/ Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
November	3.27 (1.94)	8.42 (2.99)	33.16 (5.80)	29.68 (5.49)	14.96 (3.93)	20.23 (4.55)	16.68 (4.15)	11.72 (3.49)	11.87 (3.51)	14.56 (3.88)	8.23 (2.95)	4.72 (2.28)	**14.79 (3.75^{a})**
December	1.00 (1.23)	3.20 (1.92)	28.13 (5.35)	23.15 (4.86)	13.96 (3.8)	22.39 (4.78)	12.95 (3.67)	9.23 (3.12)	11.20 (3.42)	12.40 (3.59)	5.98 (2.55)	2.73 (1.79)	**12.19 (3.34^{a})**
Mean**	**2.14 (1.58^{d})**	**5.81 (2.45^{d})**	**30.65 (5.58^{b})**	**26.42 (5.18^{a})**	**14.46 (3.87^{c})**	**21.31 (4.67^{d})**	**14.82 (3.91^{d})**	**10.48 (3.31^{d})**	**11.53 (3.47^{c})**	**13.48 (3.74^{d})**	**7.11 (2.75^{d})**	**3.72 (2.04^{d})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05)	Months (M)	: 0.118	SEm±	Months (M)	: 0.044
	Time (T)	: 0.289		Time (T)	: 0.109
	MxT	: 0.435		MxT	: 0.154

1000 h and again between 1100 to 1200 h with mean number of bees/5 min/h 30.65, 26.42 and 21.31 respectively. Thereafter it again showed a slight peak during 1500 to 1600 h with 13.48 bees/5 min/h and declined until 1800 h. More activity was observed during the month of November with a mean of 14.79 bees/5 min/h compared to 12.19 in December.

4.8.1.2 During dry season (January to May)

The hive activity was observed to be the highest as compared to other seasons (Table 17). Maximum activity was during 0900 to 1000 h with a mean number of 43.97 bees/5min/h and minimum during 0600 to 0700 h with 4.82 bees/5min/h. The activity consistently declined after 1100 h with a slight peak during 1400 to 1500 h and reduced to 11.42 bees/5min/h during 1700-1800 h. The month of April had maximum activity followed by March with a mean of 33.94 and 28.81 bees/5min/h respectively.

4.8.1.3 During southwest monsoon season (June to August)

The activity was continual throughout the days in every month in the season (Table 18). The lowest activity of day was observed during 0600 to 0700 h after which a peak activity was recorded from 1000 to 1100 h in which mean of 24.15 bees/5 min/h was observed. This was followed by a mean of 23.74 bees/5 min/h during 1100 to 1200 h and 22.30 bees/5 min/h during 1200 to 1300 h. The activity reduced in afternoon hours to reach a slight peak during 1500 to 1600 h then reduced to low activity during 1700 to 1800 h which was on par with morning hour. The activity level of foragers was almost similar in all three months with a highest mean value of 15.53 bees/5 min/h in the month of June.

4.8.2 Activity of incoming foragers with pollen

4.8.2.1 During northeast monsoon (November to December)

The maximum number of incoming bees with pollen was observed for the month November which was a mean of 5.82 bees/5 min/h. During an individual

Table 17. Foraging activity of outgoing foragers in dry season

Months↓/Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
January	1.00 (1.23)	1.42 (1.39)	11.79 (3.51)	27.38 (5.28)	24.16 (4.97)	11.30 (3.44)	19.42 (4.46)	15.90 (4.05)	8.06 (2.93)	7.87 (2.89)	7.83 (2.87)	5.77 (2.50)	**11.82(3.29^{c})**
February	1.60 (1.45)	18.41 (4.35)	56.84 (7.57)	48.51 (7.00)	31.21 (5.63)	30.64 (5.58)	27.48 (5.29)	16.52 (4.13)	17.71 (4.27)	13.83 (3.79)	18.01 (4.30)	7.48 (2.83)	**24.02(4.68^{b})**
March	1.00 (1.23)	30.45 (5.56)	34.97 (5.96)	48.93 (7.03)	56.08 (7.52)	41.56 (6.49)	31.96 (5.69)	22.09 (4.75)	27.06 (5.25)	24.27 (4.98)	15.65 (4.02)	11.66 (3.49)	**28.81(5.16^{b})**
April	10.73 (3.35)	32.89 (5.78)	49.84 (7.09)	55.03 (7.45)	45.77 (6.80)	38.40 (6.24)	34.61 (5.93)	33.61 (5.84)	32.93 (5.78)	26.86 (5.23)	27.71 (5.31)	18.90 (4.41)	**33.94(5.76^{a})**
May	9.75 (3.20)	25.32 (5.08)	29.12 (5.44)	39.99 (6.36)	29.08 (5.44)	30.24 (5.54)	24.30 (4.98)	14.48 (3.87)	25.10 (5.06)	19.53 (4.48)	14.77 (3.91)	13.32 (3.72)	**22.92(4.76^{b})**
Mean**	**4.82 (2.09^{g})**	**21.69 (4.43^{e})**	**36.51 (5.91^{abc})**	**43.97 (6.62^{a})**	**37.26 (6.07^{ab})**	**30.43 (5.45^{bc})**	**27.55 (5.27^{cd})**	**20.52 (4.52^{de})**	**22.17 (4.65^{de})**	**18.47 (4.27^{e})**	**16.79 (4.08^{ef})**	**11.42 (3.38^{f})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.508
Time (T) : 0.788
MxT :

SEm± Months (M) : 0.177
Time (T) : 0.275
MxT : 0.615

Table 18. Foraging activity of outgoing foragers in southwest monsoon season

Months↓/ Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
June	7.15 (2.77)	10.92 (3.38)	21.71 (4.71)	24.47 (4.99)	20.63 (4.59)	19.19 (4.44)	15.01 (3.94)	15.82 (4.04)	12.57 (3.62)	18.15 (4.32)	16.15 (4.1)	4.56 (2.25)	**15.53 (3.92^{a})**
July	4.52 (2.24)	4.60 (2.26)	13.92 (3.79)	20.69 (4.60)	23.86 (4.94)	28.38 (5.37)	21.34 (4.67)	14.86 (3.92)	12.35 (3.59)	15.04 (3.94)	9.03 (3.1)	5.83 (2.51)	**14.53 (3.74^{a})**
August	1.00 (1.23)	3.42 (1.98)	11.83 (3.51)	17.47 (4.24)	27.94 (5.33)	23.65 (4.91)	30.57 (5.57)	21.07 (4.64)	11.46 (3.46)	16.54 (4.13)	9.31 (3.13)	6.53 (2.65)	**15.07 (3.73^{a})**
Mean**	**4.22 (2.08^{d})**	**6.31 (2.54^{bcd})**	**15.82 (4.00^{ab})**	**20.88 (4.61^{a})**	**24.15 (4.95^{abc})**	**23.74 (4.91^{bcd})**	**22.30 (4.73^{abc})**	**17.25 (4.20^{bc})**	**12.13 (3.55^{cd})**	**16.57 (4.13^{abc})**	**11.50 (3.43^{abc})**	**5.64 (2.47^{cd})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.209
Time (T) : 0.419
MxT :

SEm± Months (M) : 0.174
Time (T) : 0.347
MxT : 0.602

day, lowest activity was recorded between 0600 to 0700 h and 1700 to 1800 h with means of 1.00 and 1.11 bees/5 min/h respectively (Table 19). The activity of foragers increased constantly from 0800 h and touched a peak at 0900 to 1000 h (16.03 bees/5 min/h). A decline followed by a small peak was observed at 1500 h and then gradual decrease until dawn was noted.

4.7.2.2 During dry season (January to May)

The maximum activity of the season was in the month of April (8.74 bees/5 min/h) which was followed by the months of February and March with 7.92 and 7.63 bees/5 min/h respectively. The minimum activity was in May with only a mean of 3.34 bees/5 min/h which was significantly low (Table 20). The lowest number of bees observed was from 0600 to 0700 h while the highest level of activity was observed during 0900 to 1000 h with a mean of 17.34 bees/5 min/h. The activity increased from 0800 h to reach the peak and then decreased till 1800 h gradually.

4.7.2.3 During southwest monsoon season (June to August)

The activity of incoming foragers with pollen was comparatively less in all months of the season (Table 21). The peak activity was shown during the month of August with a mean of 5.62 bees/5 min/h. The month of June showed relatively low activity with a mean of 2.52 bees/5 min/h. The lowest activity during an individual day was observed during early morning from 0600 to 0700 h and late evening from 1700 to 1800 h with mean of 1.16 and 1.23 bees/5 min/h respectively. Activity gradually increased from 0800 h and reached maximum between 1100 and 1200 h with mean 8.19 bees/5 min/h and then decreased until 1500 h to show a small peak during the hour.

4.8.3 Activity of incoming foragers without pollen

4.8.3.1 During northeast monsoon season (November to December)

The activity of incoming foragers without pollen was observed in every hour of the day in all months (Table 22). The activity was highest during 1200 to 1300

Table 19. Foraging activity of incoming foragers with pollen in northeast monsoon season

Months↓/Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
November	1.00 (1.23)	2.39 (1.7)	15.72 (4.03)	18.74 (4.39)	13.56 (3.75)	7.15 (2.77)	2.90 (1.85)	2.70 (1.79)	1.47 (1.40)	2.18 (1.64)	1.00 (1.23)	1.00 (1.23)	**5.82(2.24^{a})**
December	1.00 (1.23)	1.47 (1.31)	13.73 (3.77)	13.33 (3.72)	9.42 (3.15)	5.98 (2.55)	3.97 (2.12)	3.92 (2.10)	2.24 (1.65)	2.09 (1.61)	1.47 (1.40)	1.23 (1.31)	**4.99(2.1^{a})**
Mean**	**1.00 (1.22^{g})**	**1.93 (1.50^{ef})**	**14.72 (3.9^{a})**	**16.03 (4.05^{a})**	**11.49 (3.45^{b})**	**6.56 (2.65^{c})**	**3.44 (1.98^{d})**	**3.31 (1.94^{d})**	**1.85 (1.52^{ef})**	**2.14 (1.62^{e})**	**1.23 (1.31^{fg})**	**1.11 (1.26^{g})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05)	Months (M)	: 0.133	SEm±	Months (M)	: 0.039
	Time (T)	: 0.325		Time (T)	: 0.096
	MxT	: 0.385		MxT	: 0.136

Table 20. Foraging activity of incoming foragers with pollen in dry season

Months↓/Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
January	1.00 (1.23)	1.00 (1.23)	4.42 (2.22)	14.84 (3.92)	10.82 (3.37)	4.55 (2.25)	3.39 (1.97)	3.08 (1.89)	2.39 (1.7)	3.10 (1.9)	2.83 (1.83)	2.35 (1.68)	**4.48(2.09^{b})**
February	1.00 (1.23)	3.42 (1.98)	19.30 (4.45)	23.76 (4.93)	11.90 (3.52)	13.07 (3.68)	4.89 (2.32)	6.22 (2.59)	4.66 (2.27)	3.57 (2.02)	1.87 (1.54)	1.42 (1.39)	**7.92(2.65^{a})**
March	1.00 (1.23)	3.46 (1.99)	9.48 (3.16)	17.70 (4.27)	23.94 (4.94)	12.68 (3.63)	8.47 (2.99)	4.85 (2.31)	2.77 (1.81)	2.69 (1.79)	2.39 (1.7)	2.09 (1.61)	**7.63(2.61^{a})**
April	1.47 (1.4)	3.62 (2.03)	18.10 (4.31)	25.26 (5.08)	21.43 (4.68)	10.87 (3.37)	6.10 (2.57)	6.22 (2.59)	4.47 (2.23)	3.21 (1.93)	2.51 (1.73)	1.60 (1.45)	**8.74(2.78^{a})**
May	1.00 (1.23)	4.81 (2.3)	7.54 (2.84)	5.15 (2.38)	4.49 (2.23)	4.08 (2.14)	2.90 (1.85)	3.08 (1.89)	2.39 (1.7)	1.68 (1.48)	1.95 (1.57)	1.00 (1.23)	**3.34(1.90^{b})**
Mean**	**1.09 (1.26^{f})**	**3.26 (1.90^{de})**	**11.77 (3.39^{bc})**	**17.34 (4.11^{a})**	**14.52 (3.75^{ab})**	**9.05 (3.01^{c})**	**5.15 (2.34^{d})**	**4.69 (2.25^{d})**	**3.34 (1.94^{de})**	**2.85 (1.82^{de})**	**2.31 (1.67^{ef})**	**1.69 (1.47^{ef})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05)	Months (M)	: 0.430	SEm±	Months (M)	: 0.143
	Time (T)	: 0.667		Time (T)	: 0.221
	MxT	:		MxT	: 0.495

Table 21. Foraging activity of incoming foragers with pollen in southwest monsoon season

Months↓/ Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
June	1.47 (1.4)	3.00 (1.87)	4.85 (2.31)	3.55 (2.01)	3.49 (1.99)	2.99 (1.87)	1.47 (1.4)	2.09 (1.61)	1.68 (1.48)	2.49 (1.73)	2.15 (1.63)	1.00 (1.23)	**2.52(1.71^{b})**
July	1.00 (1.23)	1.68 (1.48)	2.84 (1.83)	6.19 (2.59)	7.62 (2.85)	8.87 (3.06)	7.78 (2.88)	3.98 (2.12)	2.77 (1.81)	2.49 (1.73)	1.77 (1.51)	1.47 (1.4)	**4.04(2.04^{a})**
August	1.00 (1.23)	1.42 (1.39)	7.50 (2.83)	8.04 (2.92)	9.04 (3.09)	12.71 (3.63)	9.59 (3.18)	8.66 (3.03)	2.21 (1.65)	3.90 (2.1)	2.15 (1.63)	1.23 (1.31)	**5.62(2.33^{a})**
Mean**	**1.16 (1.28^{e})**	**2.03 (1.58^{de})**	**5.06 (2.32^{abc})**	**5.93 (2.50^{ab})**	**6.71 (2.64^{ab})**	**8.19 (2.85^{a})**	**6.28 (2.48^{ab})**	**4.91 (2.25^{bc})**	**2.22 (1.64^{de})**	**2.96 (1.85^{cd})**	**2.02 (1.59^{de})**	**1.23 (1.31^{de})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05)	Months (M)	: 0.333	SEm±	Months (M)	: 0.107
	Time (T)	: 0.667		Time (T)	: 0.214
	MxT	:		MxT	: 0.37

Table 22. Foraging activity of incoming foragers without pollen in northeast monsoon season

Months↓/Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
November	1.68 (1.48)	6.75 (2.69)	5.67 (2.48)	8.66 (3.03)	11.67 (3.49)	15.43 (3.99)	16.96 (4.18)	13.67 (3.76)	15.68 (4.02)	12.19 (3.56)	9.47 (3.16)	6.99 (2.74)	**10.40(3.21^{a})**
December	1.00 (1.23)	2.48 (1.73)	5.56 (2.46)	8.23 (2.96)	9.39 (3.15)	17.22 (4.21)	17.73 (4.27)	11.71 (3.49)	10.97 (3.39)	10.17 (3.27)	12.21 (3.57)	5.98 (2.55)	**9.39(3.02^{b})**
Mean**	**1.34 (1.35^{g})**	**4.61 (2.21^{f})**	**5.61 (2.47^{ef})**	**8.44 (2.99^{d})**	**10.53 (3.31^{c})**	**16.32 (4.1^{a})**	**17.34 (4.22^{a})**	**12.69 (3.63^{bc})**	**13.33 (3.70^{b})**	**11.18 (3.41^{bc})**	**10.84 (3.36^{c})**	**6.48 (2.64^{e})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.138
Time (T) : 0.34
MxT : 0.446

SEm± Months (M) : 0.046
Time (T) : 0.112
MxT : 0.158

h with a mean number of 17.34 bees/5 min/h and lowest during 0600 to 0700 h with a mean of 1.34 bees/5 min/h. The activity constantly increased from morning hours to reach the peak and then decreased continuously till late evening. The month of November showed the highest activity with 10.43 bees/5 min/h as compared to a 9.39 bees/5 min/h in the month of December.

4.7.3.2 During dry season (January to May)

The number of incoming foragers was comparatively high during the whole season. The lowest activity of a day was recorded between 0600 to 0700 h after which it abruptly increased to a 15.76 bees/5 min/h (Table 23). It continued to increase progressively to reach a peak at 1000 to 1100 h where average of 28.50 bees/5 min/h was observed. Thereafter a steady decline in activity was recorded until 1800 h. The number of foragers was considerably higher in the month of April with a mean of 26.94 bees/5 min/h. Lowest activity of all months was mean number of 9.05 bees/5 min/h during month January. Level of activity observed during the months of March (22.62 bees/5 min/h) and May (21.23 bees/5 min/h) were comparable.

4.7.3.3 During southwest monsoon season (June to August)

The incoming of foragers without pollen was noticed throughout the day in the season (Table 24). Lowest activity was observed in early morning from 0600 to 0700 h and it increased tremendously from 0800 h to reach a peak at 1000 to 1100 h of 18.18 bees/5 min/h. It remained almost constant until 1300 h (18.78 bees/5 min/h) after which it decreased till evening hours. The number of bees remained almost identical throughout the months with a highest value of 13.98 bees/5 min/h in the month of June.

4.7.4 Activity of foragers regardless of the season

4.7.4.1 Foraging activity of outgoing foragers

Table 23. Foraging activity of incoming foragers without pollen in dry season

Months↓/ Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
January	1.00 (1.23)	1.00 (1.23)	5.41 (2.43)	15.42 (3.99)	13.47 (3.74)	11.51 (3.47)	18.44 (4.35)	10.90 (3.38)	9.04 (3.09)	9.75 (3.2)	7.36 (2.8)	5.28 (2.40)	**9.05 (2.94^{c})**
February	1.42 (1.39)	14.36 (3.86)	31.83 (5.69)	28.35 (5.37)	22.86 (4.83)	27.91 (5.33)	21.36 (4.68)	19.73 (4.49)	17.03 (4.19)	18.28 (4.33)	19.28 (4.45)	9.35 (3.14)	**19.31(4.31^{b})**
March	1.00 (1.23)	25.28 (5.08)	23.06 (4.85)	25.83 (5.13)	38.56 (6.25)	30.07 (5.53)	23.69 (4.92)	21.93 (4.74)	24.55 (5.01)	22.63 (4.81)	17.55 (4.25)	17.36 (4.23)	**22.62(4.66^{b})**
April	7.11 (2.76)	20.98 (4.64)	27.06 (5.25)	32.64 (5.76)	41.28 (6.46)	35.52 (6.00)	30.55 (5.57)	30.40 (5.56)	24.90 (5.04)	26.22 (5.17)	22.78 (4.83)	23.83 (4.93)	**26.94(5.16^{a})**
May	4.45 (2.23)	17.20 (4.21)	22.33 (4.78)	30.83 (5.59)	26.34 (5.18)	31.94 (5.69)	29.06 (5.44)	25.56 (5.11)	23.03 (4.85)	18.93 (4.41)	15.52 (4.00)	9.54 (3.17)	**21.23(4.55^{b})**
Mean**	**3.00 (1.76^{f})**	**15.76 (3.8^{de})**	**21.94 (4.6^{abc})**	**26.61 (5.16^{a})**	**28.50 (5.29^{a})**	**27.39 (5.20^{a})**	**24.62 (4.99^{ab})**	**21.70 (4.65^{abc})**	**19.71 (4.43^{bcd})**	**19.16 (4.38^{bcd})**	**16.50 (4.06^{cde})**	**13.07 (3.57^{e})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.478
Time (T) : 0.741
MxT :

SEm± Months (M) : 0.165
Time (T) : 0.256
MxT : 0.572

Table 24. Foraging activity of incoming foragers without pollen in southwest monsoon season

Months↓/ Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
June	4.49 (2.23)	9.89 (3.22)	14.90 (3.92)	21.19 (4.66)	19.13 (4.43)	14.08 (3.82)	11.89 (3.52)	17.58 (4.25)	15.76 (4.03)	17.28 (4.22)	15.27 (3.97)	6.30 (2.61)	**13.98(3.74^{a})**
July	1.68 (1.48)	5.01 (2.35)	7.33 (2.79)	13.27 (3.71)	20.55 (4.59)	21.25 (4.66)	19.89 (4.52)	19.69 (4.49)	16.58 (4.13)	11.31 (3.44)	9.12 (3.1)	6.38 (2.62)	**12.67(3.49^{a})**
August	1.00 (1.23)	2.69 (1.79)	9.51 (3.16)	11.15 (3.41)	14.85 (3.92)	18.90 (4.41)	22.86 (4.83)	19.08 (4.43)	12.59 (3.62)	16.80 (4.16)	12.15 (3.56)	10.63 (3.34)	**12.68(3.49^{a})**
Mean**	**2.39 (1.65^{e})**	**5.86 (2.45^{de})**	**10.58 (3.30^{bcd})**	**15.20 (3.93^{ab})**	**18.18 (4.31^{a})**	**18.08 (4.29^{a})**	**18.21 (4.29^{a})**	**18.78 (4.39^{a})**	**14.98 (3.93^{ab})**	**15.13 (3.94^{ab})**	**12.18 (3.54^{abc})**	**7.77 (2.85^{cd})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)

(Values in the parenthesis are square root transformed values)

CD (0.05)	Months (M)	: 0.514	SEm±	Months (M)	: 0.172
	Time (T)	: 1.028		Time (T)	: 0.345
	MxT	:		MxT	: 0.597

The activity of outgoing foragers irrespective of season is represented in Table 25. The comparison of observations shows that the highest number of outgoing bees was recorded at 0900 to 1000 h (33.59 bees/5 min/h) followed by 0800 to 0900 h (29.13 bees/5 min/h), 1000 to 1100 h (28.77 bees/5 min/h) and 1100 to 1200 h (26.60 bees/5 min/h). Significantly lower activity was shown during 0600 to 0700 h with mean of 4.10 bees/5 min/h. Maximum activity was in the month of April with mean 33.94 bees/5 min/h which was significantly different from all other months. It is followed by March (28.81 bees/5 min/h) and February (24.02 bees/5 min/h) both of which differ significantly. Least activity was observed in January with a mean 11.82 bees/5 min/h which was on par with December (12.19 bees/5 min/h).

4.7.4.2 Foraging activity of incoming foragers with pollen

The activity of incoming foragers with pollen irrespective of season is represented in Table 26. The highest activity was observed in April with a mean of 8.74 bees/5 min/h which was on par with the month of February (7.92 bees/5 min/h). Lowest activity of incoming foragers with pollen was recorded in June (2.52 bees/5 min/h) which was on par with May and July with mean of 3.34 and 4.04 bees/5 min/h respectively. The lowest activity in an individual day was observed during early morning from 0600 to 0700 h (1.09 bees/5 min/h). The activity increased from 0800 h to reach a peak between 0900 to 1000 h (13.65 bees/5 min/h) which was on par with 11.57 bees/5 min/h during 1000 to 1100 h. Thereafter the activity reduced continuously until late evening.

4.7.4.3 Foraging activity of incoming foragers without pollen

The activity of bees returning without pollen irrespective of season is given in Table 27. The month of April saw the maximum number of 26.94 bees/5 min/h which was significantly different from months that followed, *viz*., March (22.62 bees/5 min/h) and May (21.23 bees/5 min/h), which were both on par. The lowest activity was observed in December (9.39 bees/5 min/h) and January (9.05 bees/5 min/h) both of which are on par. The highest activity of the day was recorded from

Table 25. Foraging activity of outgoing foragers irrespective of season

Months↓/ Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
November	3.27 (1.94)	8.42 (2.99)	33.16 (5.80)	29.68 (5.49)	14.96 (3.93)	20.23 (4.55)	16.68 (4.15)	11.72 (3.49)	11.87 (3.51)	14.56 (3.88)	8.23 (2.95)	4.72 (2.28)	**14.79(3.75^{de})**
December	1.00 (1.23)	3.20 (1.92)	28.13 (5.35)	23.15 (4.86)	13.96 (3.8)	22.39 (4.78)	12.95 (3.67)	9.23 (3.12)	11.20 (3.42)	12.40 (3.59)	5.98 (2.55)	2.73 (1.79)	**12.19(3.34^{e})**
January	1.00 (1.23)	1.42 (1.39)	11.79 (3.51)	27.38 (5.28)	24.16 (4.97)	11.30 (3.44)	19.42 (4.46)	15.90 (4.05)	8.06 (2.93)	7.87 (2.89)	7.83 (2.87)	5.77 (2.50)	**11.82(3.29^{e})**
February	1.60 (1.45)	18.41 (4.35)	56.84 (7.57)	48.51 (7.00)	31.21 (5.63)	30.64 (5.58)	27.48 (5.29)	16.52 (4.13)	17.71 (4.27)	13.83 (3.79)	18.01 (4.30)	7.48 (2.83)	**24.02(4.68^{c})**
March	1.00 (1.23)	30.45 (5.56)	34.97 (5.96)	48.93 (7.03)	56.08 (7.52)	41.56 (6.49)	31.96 (5.69)	22.09 (4.75)	27.06 (5.25)	24.27 (4.98)	15.65 (4.02)	11.66 (3.49)	**28.81(5.16^{b})**
April	10.73 (3.35)	32.89 (5.78)	49.84 (7.09)	55.03 (7.45)	45.77 (6.80)	38.40 (6.24)	34.61 (5.93)	33.61 (5.84)	32.93 (5.78)	26.86 (5.23)	27.71 (5.31)	18.90 (4.41)	**33.94(5.77^{a})**
May	9.75 (3.20)	25.32 (5.08)	29.12 (5.44)	39.99 (6.36)	29.08 (5.44)	30.24 (5.54)	24.30 (4.98)	14.48 (3.87)	25.10 (5.06)	19.53 (4.48)	14.77 (3.91)	13.32 (3.72)	**22.92(4.76^{bc})**
June	7.15 (2.77)	10.92 (3.38)	21.71 (4.71)	24.47 (4.99)	20.63 (4.59)	19.19 (4.44)	15.01 (3.94)	15.82 (4.04)	12.57 (3.62)	18.15 (4.32)	16.15 (4.1)	4.56 (2.25)	**15.53(3.93^{d})**
July	4.52 (2.24)	4.60 (2.26)	13.92 (3.79)	20.69 (4.60)	23.86 (4.94)	28.38 (5.37)	21.34 (4.67)	14.86 (3.92)	12.35 (3.59)	15.04 (3.94)	9.03 (3.1)	5.83 (2.51)	**14.53(3.74^{de})**
August	1.00 (1.23)	3.42 (1.98)	11.83 (3.51)	17.47 (4.24)	27.94 (5.33)	23.65 (4.91)	30.57 (5.57)	21.07 (4.64)	11.46 (3.46)	16.54 (4.13)	9.31 (3.13)	6.53 (2.65)	**15.07(3.73^{de})**
Mean**	**4.10 (1.98^{g})**	**13.90 (3.47^{e})**	**29.13 (5.27^{ab})**	**33.53 (5.73^{a})**	**28.77 (5.29^{ab})**	**26.60 (5.13^{b})**	**23.43 (4.83^{b})**	**17.53 (4.18^{c})**	**17.03 (4.09^{cd})**	**16.90 (4.12^{cd})**	**13.27 (3.62^{de})**	**8.15 (2.84^{f})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)
(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.469
Time (T) : 0.514
MxT :

SEm± Months (M) : 0.168
Time (T) : 0.185
MxT : 0.584

Table 26. Foraging activity of incoming foragers with pollen irrespective of season

Months↓/Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	**0700**	**0800**	**0900**	**1000**	**1100**	**1200**	**1300**	**1400**	**1500**	**1600**	**1700**	
November	1.00 (1.23)	2.39 (1.7)	15.72 (4.03)	18.74 (4.39)	13.56 (3.75)	7.15 (2.77)	2.90 (1.85)	2.70 (1.79)	1.47 (1.40)	2.18 (1.64)	1.00 (1.23)	1.00 (1.23)	**5.82(2.25^{d})**
December	1.00 (1.23)	1.47 (1.31)	13.73 (3.77)	13.33 (3.72)	9.42 (3.15)	5.98 (2.55)	3.97 (2.12)	3.92 (2.10)	2.24 (1.65)	2.09 (1.61)	1.47 (1.40)	1.23 (1.31)	**4.99(2.16^{d})**
January	1.00 (1.23)	1.00 (1.23)	4.42 (2.22)	14.84 (3.92)	10.82 (3.37)	4.55 (2.25)	3.39 (1.97)	3.08 (1.89)	2.39 (1.7)	3.10 (1.9)	2.83 (1.83)	2.35 (1.68)	**4.48(2.09^{de})**
February	1.00 (1.23)	3.42 (1.98)	19.30 (4.45)	23.76 (4.93)	11.90 (3.52)	13.07 (3.68)	4.89 (2.32)	6.22 (2.59)	4.66 (2.27)	3.57 (2.02)	1.87 (1.54)	1.42 (1.39)	**7.92(2.66^{ab})**
March	1.00 (1.23)	3.46 (1.99)	9.48 (3.16)	17.70 (4.27)	23.94 (4.94)	12.68 (3.63)	8.47 (2.99)	4.85 (2.31)	2.77 (1.81)	2.69 (1.79)	2.39 (1.7)	2.09 (1.61)	**7.63(2.62^{bc})**
April	1.47 (1.4)	3.62 (2.03)	18.10 (4.31)	25.26 (5.08)	21.43 (4.68)	10.87 (3.37)	6.10 (2.57)	6.22 (2.59)	4.47 (2.23)	3.21 (1.93)	2.51 (1.73)	1.60 (1.45)	**8.74(2.78^{a})**
May	1.00 (1.23)	4.81 (2.3)	7.54 (2.84)	5.15 (2.38)	4.49 (2.23)	4.08 (2.14)	2.90 (1.85)	3.08 (1.89)	2.39 (1.7)	1.68 (1.48)	1.95 (1.57)	1.00 (1.23)	**3.34(1.90^{ef})**
June	1.47 (1.4)	3.00 (1.87)	4.85 (2.31)	3.55 (2.01)	3.49 (1.99)	2.99 (1.87)	1.47 (1.4)	2.09 (1.61)	1.68 (1.48)	2.49 (1.73)	2.15 (1.63)	1.00 (1.23)	**2.52(1.71^{f})**
July	1.00 (1.23)	1.68 (1.48)	2.84 (1.83)	6.19 (2.59)	7.62 (2.85)	8.87 (3.06)	7.78 (2.88)	3.98 (2.12)	2.77 (1.81)	2.49 (1.73)	1.77 (1.51)	1.47 (1.4)	**4.04(2.04^{def})**
August	1.00 (1.23)	1.42 (1.39)	7.50 (2.83)	8.04 (2.92)	9.04 (3.09)	12.71 (3.63)	9.59 (3.18)	8.66 (3.03)	2.21 (1.65)	3.90 (2.1)	2.15 (1.63)	1.23 (1.31)	**5.62(2.33^{bcd})**
Mean**	**1.09 (1.26^{f})**	**2.63 (1.73^{ef})**	**10.35 (3.17^{bc})**	**13.65 (3.62^{a})**	**11.57 (3.36^{ab})**	**8.30 (2.89^{c})**	**5.15 (2.31^{d})**	**4.48 (2.19^{d})**	**2.70 (1.77^{e})**	**2.74 (1.79^{e})**	**2.01 (1.57^{ef})**	**1.44 (1.38^{ef})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)
(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.336
Time (T) : 0.368
MxT : 1.162

SEm± Months (M) : 0.121
Time (T) : 0.132
MxT : 0.418

Table 27. Foraging activity of incoming foragers without pollen irrespective of season

Months↓/Hours→	Mean number of bees/5 minutes/colony*												Mean**
	0600	0700	0800	0900	1000	1100	1200	1300	1400	1500	1600	1700	
November	1.68 (1.48)	6.75 (2.69)	5.67 (2.48)	8.66 (3.03)	11.67 (3.49)	15.43 (3.99)	16.96 (4.18)	13.67 (3.76)	15.68 (4.02)	12.19 (3.56)	9.47 (3.16)	6.99 (2.74)	**10.40(3.21^{de})**
December	1.00 (1.23)	2.48 (1.73)	5.56 (2.46)	8.23 (2.96)	9.39 (3.15)	17.22 (4.21)	17.73 (4.27)	11.71 (3.49)	10.97 (3.39)	10.17 (3.27)	12.21 (3.57)	5.98 (2.55)	**9.39(3.02^{e})**
January	1.00 (1.23)	1.00 (1.23)	5.41 (2.43)	15.42 (3.99)	13.47 (3.74)	11.51 (3.47)	18.44 (4.35)	10.90 (3.38)	9.04 (3.09)	9.75 (3.2)	7.36 (2.8)	5.28 (2.40)	**9.05(2.94^{e})**
February	1.42 (1.39)	14.36 (3.86)	31.83 (5.69)	28.35 (5.37)	22.86 (4.83)	27.91 (5.33)	21.36 (4.68)	19.73 (4.49)	17.03 (4.19)	18.28 (4.33)	19.28 (4.45)	9.35 (3.14)	**19.31(4.31^{b})**
March	1.00 (1.23)	25.28 (5.08)	23.06 (4.85)	25.83 (5.13)	38.56 (6.25)	30.07 (5.53)	23.69 (4.92)	21.93 (4.74)	24.55 (5.01)	22.63 (4.81)	17.55 (4.25)	17.36 (4.23)	**22.62(4.67^{b})**
April	7.11 (2.76)	20.98 (4.64)	27.06 (5.25)	32.64 (5.76)	41.28 (6.46)	35.52 (6.00)	30.55 (5.57)	30.40 (5.56)	24.90 (5.04)	26.22 (5.17)	22.78 (4.83)	23.83 (4.93)	**26.94(5.16^{a})**
May	4.45 (2.23)	17.20 (4.21)	22.33 (4.78)	30.83 (5.59)	26.34 (5.18)	31.94 (5.69)	29.06 (5.44)	25.56 (5.11)	23.03 (4.85)	18.93 (4.41)	15.52 (4.00)	9.54 (3.17)	**21.23(4.55^{b})**
June	4.49 (2.23)	9.89 (3.22)	14.90 (3.92)	21.19 (4.66)	19.13 (4.43)	14.08 (3.82)	11.89 (3.52)	17.58 (4.25)	15.76 (4.03)	17.28 (4.22)	15.27 (3.97)	6.30 (2.61)	**13.98(3.74^{c})**
July	1.68 (1.48)	5.01 (2.35)	7.33 (2.79)	13.27 (3.71)	20.55 (4.59)	21.25 (4.66)	19.89 (4.52)	19.69 (4.49)	16.58 (4.13)	11.31 (3.44)	9.12 (3.1)	6.38 (2.62)	**12.67(3.49^{cd})**
August	1.00 (1.23)	2.69 (1.79)	9.51 (3.16)	11.15 (3.41)	14.85 (3.92)	18.90 (4.41)	22.86 (4.83)	19.08 (4.43)	12.59 (3.62)	16.80 (4.16)	12.15 (3.56)	10.63 (3.34)	**12.68(3.49^{cd})**
Mean**	**2.48 (1.64^{f})**	**10.56 (3.08^{e})**	**15.27 (3.78^{d})**	**19.56 (4.36^{abc})**	**21.81 (4.60^{ab})**	**22.38 (4.71^{a})**	**21.24 (4.63^{a})**	**19.02 (4.37^{abc})**	**17.01 (4.13^{bcd})**	**16.36 (4.05^{cd})**	**14.07 (3.77^{d})**	**10.16 (3.17^{e})**	

***** Mean of four replications of bee colony

**Mean of months and time (mean in the columns/ rows with same alphabet do not differ significantly by DMRT at 5%)
(Values in the parenthesis are square root transformed values)

CD (0.05) Months (M) : 0.453
Time (T) : 0.496
MxT :

SEm± Months (M) : 0.163
Time (T) : 0.178
MxT : 0.564

1100 to 1200 h (22.38 bees/5 min/h) which was on par with 1000 to 1100 h (21.81 bees/5 min/h) and 1200 to 1300 h (21.24 bees/5 min/h). The least activity was observed during 0600 to 0700 h with a mean 2.48 bees/5 min/h and was significantly different from all other hours.

4.7.5 Relative incidence of incoming foragers with pollen

The relative presence of pollen foragers among the total incoming foragers was found for the study period. An average of 24.24 per cent pollen foragers was observed over the months (Table 28). The ratio of incoming pollen foragers to total incoming foragers was highest pollen foragers during November, which happened to be 4.88 pollen foragers/ 5 min/ h out of a total of 14.38 total incoming foragers/ 5 min/ h accounting for a 33.91 per cent making it significantly different from all other months. It was followed by December with a 4.04 pollen foragers/ 5 min/ h out of 12.5 incoming foragers/ 5 min/ h (32.33 per cent) which again was significantly different.

The minimum ratio of pollen foragers were observed in month May and June which was 10.75 per cent and 10.8 per cent respectively which were on par with each other. Comparatively higher and similar activity of foragers was observed during January through April, July and August which was around 30.87 to 21.77 per cent, all of which were on par with each other. The activity of pollen foragers was seen reducing from November in a steady manner until April, declining very rapidly during May and June, then increasing tremendously from July till August.

Table 28. Relative incidence of foragers incoming with pollen loads

Month	Total incoming foragers(bees/5 min/hr)*	Total pollen foragers(bees/5 min/hr)*	Ratio of PF to TIF**	% of pollen foragers
November	14.38	4.88	0.6820 (4.70^{a})	33.91
December	12.5	4.04	0.2502 (2.87^{b})	32.33
January	12.41	3.83	0.2247 (2.63^{bc})	30.87
February	27.16	7.58	0.2193 (2.60^{bc})	27.91
March	29.75	7.48	0.1742 (2.32^{bc})	25.14
April	37.25	9.46	0.1820 (2.42^{bc})	25.39
May	23.46	2.52	0.0983 (1.74^{c})	10.75
June	16	1.73	0.1315 (1.83^{c})	10.8
July	16.27	3.54	0.1627 (2.22^{bc})	21.77
August	20.20	5.69	0.1956 (2.53^{bc})	28.14
CD (0.05)	--	--	0.932	--
Average	20.938	5.075		**24.24**

*Mean values of four replications of hive

**Ratio of mean values of incoming pollen foragers to total incoming foragers

(Values in parenthesis are Arc sine transformed values)

Discussion

5. DISCUSSION

The study was conducted in the Department of Agricultural Entomology, College of Agriculture, Vellayani to identify the resources of pollen and honey of stingless bees and their foraging activity from 2017-2020. The study was conducted during three season northeast monsoon, dry and southwest monsoon seasons. Attempts have been made to discuss the salient findings in this chapter.

5.1. POLLEN IDENTIFICATION AND CHARACTERIZATION

The present study revealed that the majority of pollen types out of 92 types identified were of native plant species such as *Cocos nucifera*, *Mimosa pudica*, *Areca* sp., *Acacia catechu* etc. This is in accordance with the study conducted by Vossler (2019) in a forest region, where he reported that stingless bee collected pollen mostly from the native species, even though ornamental exotic species were abundantly present. The pollen types identified from the honey and pollen samples provided an insight into the bee flora of the eighteen locations in the study. The pollen types included trees, shrubs, herbs and climbers of which tree species were of most abundance. This can be confirmed from the study of Silva *et al*. (2019) where in spite of all climatic adversity, stingless bee collected resources from native trees, shrubs and herbaceous plants signifying the importance of all plant types in the habitat of the bee colony.

The pollen types identified from uplands were more in number and diverse in composition as compared to the midlands which might be due to more occurrence of forest ecosystems in upland regions. This agrees with the findings of Kaluza *et al*. (2017) and Machado *et al*. (2020) who stated that bees maximize intake of more plant species in natural, diverse and resource abundant habitats to secure collection of sufficient amounts and also to meet the functional needs of colonies.

The pollen reported from areas near to forest regions recorded more number of tree species like *Peltophorum pterocarpum*, *Delonix regia* and *Caesalpinia pulcherrima*. This can be confirmed from study of Absy *et al*. (2018) where they

found out that when forest area is considered, stingless bee showed more preference to flowers of trees rather than shrubs and herbs as they would be scattered. The number of weeds such as *Tridax procumbens*, *Ageratum conyzoides Mikania micrantha* etc and wild plants like *Mitracarpus hirtus* recorded were more, which might be due to their abundance in areas near to apiaries or the regional vegetative pattern especially in upland areas near to the forest. This is also supported by Absy *et al*. (2018) who stated that where there is natural distribution of vegetation, it was found that woody species from edges of crops provided food for stingless bee. Among the 93 identified species, 15 species are anemophilous in nature whose flowers are generally small and unattractive and might be visited by bees either due to accidental contamination of body of bee or abundance of pollen as a result of less competition from other bee species. The evidence for visits of anemophilous plants by bees for floral and non-floral resources has been recorded by Saunders (2018) and Sahney *et al.* (2018).

The pollen types included plant species of different habits and nature ranging from trees like *Cocos nucifera* to shrubs like *Melastoma malabathricum* to herbs and climbers like *Passiflora edulis*, *Ocimum gratissimum* etc indicating the height of the plant is not limiting factor for the stingless bees. This can be also cited in the study by Ramalho (2004) where stingless bees had a vertical distribution upto 7 m while in another study by Ciar *et al*. (2013) the most preferred height by stingless bee was reported to be 1 m, comparable upto 3 m and their diet is satisfied by preferred sources in the proximity of colonies (Basari *et al*., 2018).

5.2. DISTRIBUTION OF POLLEN CLASS TYPES OVER SEASONS

The percentage distribution of pollen types in honey and pollen samples indicated the species richness of the locations. The percentage of predominant to secondary pollen types in the pollen samples over three seasons accounted for about 20 per cent of total obtained pollen types, while in honey samples it accounted for 17.4 per cent. Minor and important minor pollen types in the pollen samples accounts for 80 per cent and while in honey samples for 82.6 per cent. This is comparable to the conclusions of Balderas (2016) that only 16 per cent of pollen

were secondary to predominant types, rest of 84 per cent constituted the minor and important minor types. Microscopic analysis of honey revealed that most of the samples were multifloral in nature similar to the finding of Rimna *et al*. (2017).

When the species richness of each season was compared 36 pollen types constituting 31.3 per cent of total identified pollen types were present in pollen samples of northeast monsoon season, whereas 34 pollen types (29.57 per cent) was recorded from dry season and 32 pollen types (27.8 per cent) recorded during southwest monsoon season. Eltz *et al*. (2001) has stated that at any time, bee colonies forage on comparatively broad range of pollen plants which is in accordance to the study. In the honey samples obtained during northeast monsoon season, 22 pollen types accounting for 19.13 per cent and during dry season, 32 pollen types constituting 27.8 per cent was recorded. Similar finding has been reported by Balderas (2016) where it is stated that diversity of pollen is directly related to blooming pattern, whereas higher species richness was during dry season.

The pollen grains of *C. nucifera*, *M. pudica* and *M. pinnata* was found to be predominant pollen type in both the honey and pollen samples over the three seasons. The importance of all three plants as relevant sources of nectar or pollen has been reported by Agashe and Rangaswamy (1997). Aside from these three pollen types, the pollen spectrum is also exhibiting the presence of wild weed species in a crucial pattern. Vijayakumar and Jeyaraaj (2016) has reported the prevalence of wild plant species like *T. procumbens* and *A. sessilis* in the results.

Tree species like *P. pterocarpum*, *M. peltata* and *S. mahagoni* were also secondary sources for the bees. This lies in agreement with the findings of Joshi *et al*. (1998) and Danaraddi (2007) who reported the importance of *P. pterocarpum* as source for stingless bees. The pollen types of family Arecaceae such as *Areca* sp. seems to be a secondary pollen type in all seasons due to the abundant and constant supply throughout the year as stated by Rech and Absy (2011b) in their study.

The preference of anemophilous plants like *B. flabellifer*, pollen types of Poaceae family which do not possess features attractive to the stingless bee is

clearly visible in all seasons indicating lack of co-relation between nectar and pollen collection. This might be due to the production of copious amounts of pollen and it might be a very crucial feature for the bees that continuously requires large quantities of pollen. The anemophilous plant species has been found to be visited by stingless bee for pollen in the study by Saunders (2018) and presence of the same in pot pollen has also been reported by Vossler (2019).

The species like *T. arjuna*, *A. spinosus*, *Acacia mangium*, *P. edulis* and members from families Euphorbiaceae, Phyllanthaceae, Caesalpiniaceae were reported as minor sources of pollen and nectar in the study. Layek and Karmakar (2018) recorded similar findings in their study where *A. auriculiformis*, *T. arjuna* were important pollen and nectar sources. The critical investigation of pollen and nectar samples showed differences in floral preference by stingless bee during three seasons.

During southwest monsoon period the percentage of weeds species were higher and were found in most of the locations which might be due to the unavailability of other crops. This is in accordance with the findings of Thakodee *et al*. (2018) that in southwest monsoon periods food source of stingless bees change from economic crops to weeds and their flowering period varied with species.

5.3. FREQUENCY OF OCCURRENCE OF POLLEN TYPES

Out of the total 115 pollen types obtained, *C. nucifera*, *M. pudica* and *M. pinnata* were of major importance to bees and were frequent during all three seasons (Table 9 & 10). This is in accordance with the studies conducted by Vijayakumar and Jeyaraaj (2016) wherein they found *C. nucifera* to be most frequent and wild species like *T. procumbens* and *A. sessilis* to be of high importance to stingless bees. The importance of *C. nucifera* as frequent pollen (Fig. 4a, 4b & 4c) has also been reported by Selvaraju *et al*. (2019) where it is stated that stingless bee have preference to *C. nucifera* as they flower throughout the year and have higher sugar concentrations and hence, higher frequency of pollen in samples.

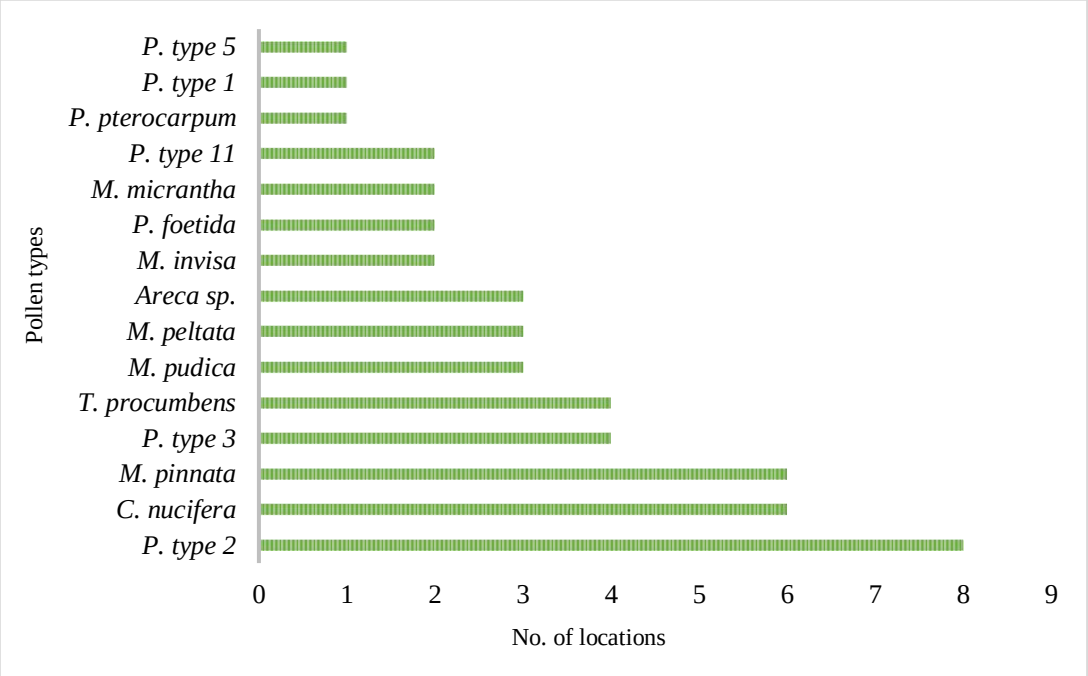


Fig 3a. Frequency of occurrence of pollen types in honey samples during northeast monsoon season

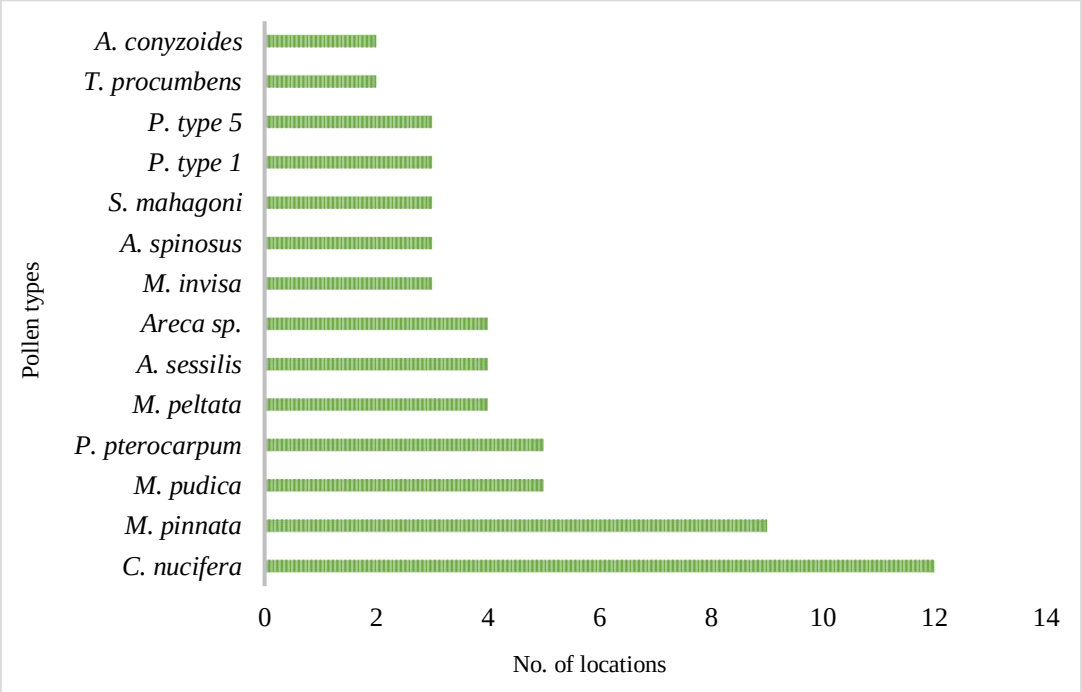


Fig 3b. Frequency of occurrence of pollen types in honey samples during dry season

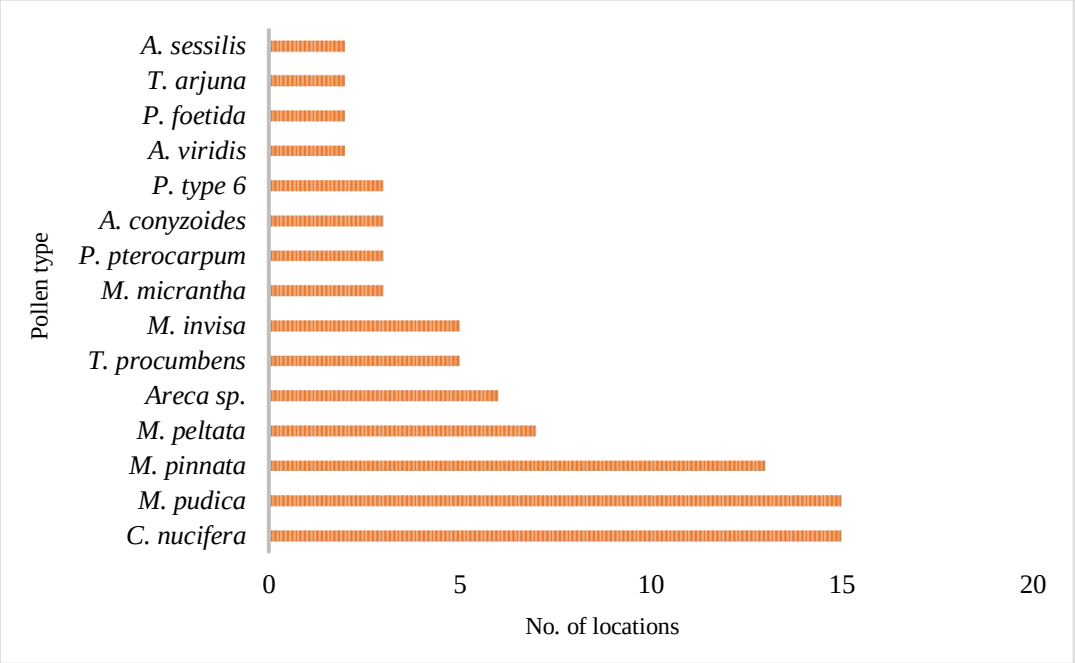


Fig 4a. Frequency of occurrence of pollen types in pollen samples during northeast monsoon season

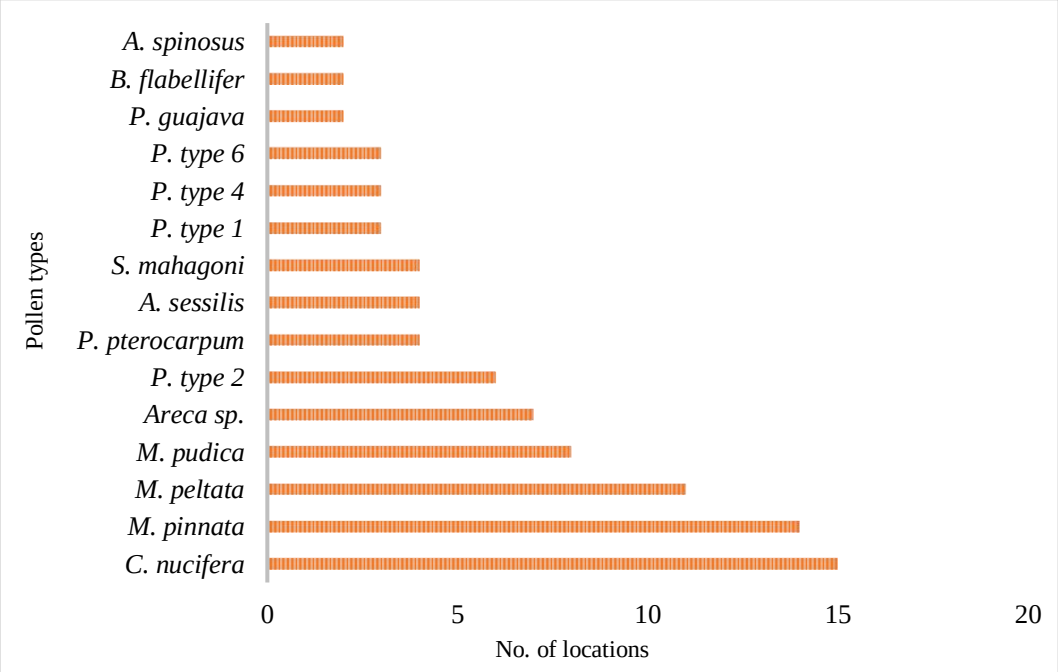


Fig 4b. Frequency of occurrence of pollen types in pollen samples during dry season

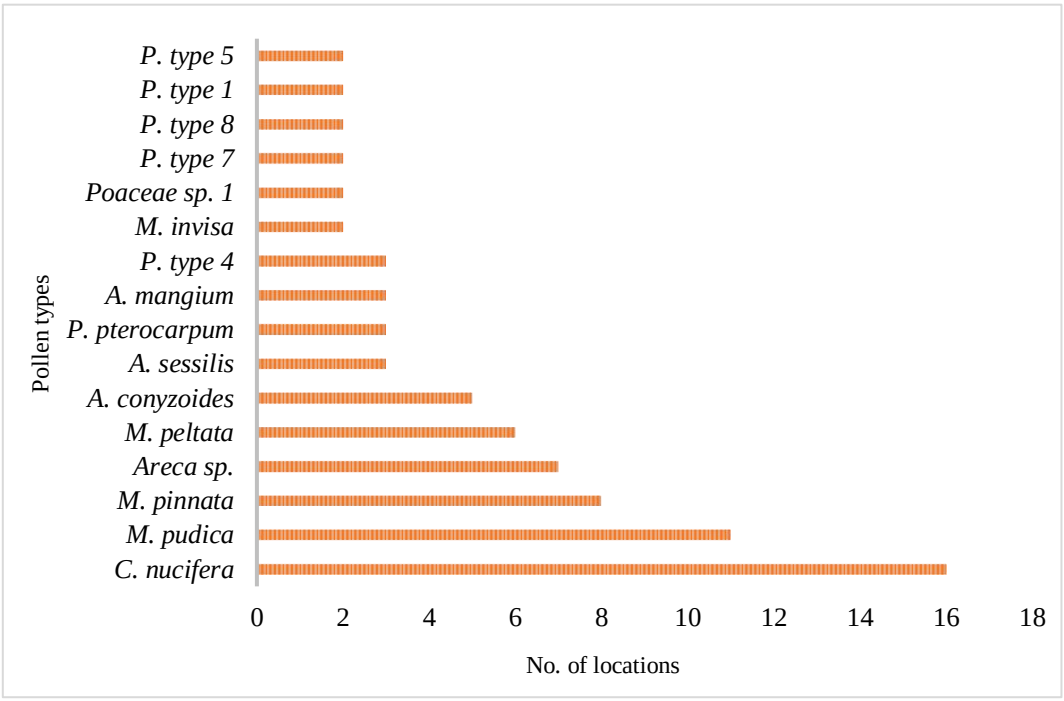


Fig 4c. Frequency of occurrence of pollen types in pollen samples during southwest monsoon season

Pollen of species like *Areca* sp., *M. peltata*, *M. diplotricha*, *T. procumbens*, *P. pterocarpum*, *A. conyzoides*, *A. sessilis*, *B. flabellifer*, *S. mahagoni* were of medium importance to the bees. These pollen appeared frequently in at least one of the seasons as significant source for forage. *M. pudica* being a nectarless plant frequently encountered in honey samples (Fig. 3a & 3b) might be because of activity of worker bees inside the hive results in mixing of pollen (Villanueva-Guitierrez *et al.*, 2009). Devender *et al*. (2019) stated in their work that *A. conyzoides*, *M. pudica* and *T. procumbens* were predominant and frequent in the honey samples obtained from the study area.

A. viridis, *A. hybridus* and member of Poaceae family were infrequent or sporadic in honey and pollen loads. The same has been recorded by Ramanujam and Kalpana (1995) in their results. Pollen of members from families Amaranthaceae, Passifloraceae, Myrtaceae, Asteraceae, Combretaceae, Phyllanthaceae and Poaceae were infrequent or rare in location. This might be due to the anemophilous nature and the accidental contamination of nectar collected by the bees. Another explanation is that strategy exhibited by bees for maintaining infidelity towards food sources might result in the infrequent or rare pollen types (Marques- Souza, 2010).

The variation in frequency of occurrence of pollen types between locations were also noticeable. *C. nucifera*, *M. pudica*, *Areca* sp., *M. pinnata* were more common in most of the locations and they bloom throughout the year explaining their continuous availability and occurrence as frequent pollen type in all seasons which is also observed by Shwetha (2013).

5.4. SEASONAL VARIATION

The analysis of pollen and honey samples provided the confirmation for variation in species and number of bee flora in different locations during three seasons (Table 11). This might be due to the difference in foraging strategy and requirements by bee colonies during different conditions. This variation in activity

of colonies according to the climate and availability of floral resources has been reported by Aleixo *et al*. (2017).

The maximum diversity in pollen types foraged was observed during dry season (78) which was followed by northeast monsoon season (67) and southwest monsoon season (62). This might be due to the fact that blooming period of most of these plants coincides with dry season, thus increasing the availability of food sources. The least pollen types observed during southwest monsoon season might be due to the hindrance in movement of bees due to the torrential rain and high wind velocity.

Pollen from plant species of *C. nucifera*, *Areca* sp., *B. flabellifer*, Arecaceae sp.1, members of Arecaceae family were available throughout the year. Jhansi *et al*. (1994) has reported *C. nucifera* to be a source throughout the year while Bhargava *et al*. (2009) reported that palms are good source of nectar. Similar findings were also made by Thakodee *et al*. (2018) who reported *A. catechu* to be secondary source throughout year. Tree species like *M. pinnata*, *M. peltata*, *P. pterocarpum*, *D. regia*, *Ceiba pentandra* were also recorded as sources throughout the three seasons. Wild species like *M. pudica*, *M. diplotricha*, *T. procumbens*, *A. conyzoides*, *Amaranthus spinosus* were present in all three seasons.

There was a continuous presence of pollen from families like Amaranthaceae, Fabaceae, Cucurbitaceae, Passifloraceae and Asteraceae throughout the year even though plant species differed with time. In a study on food source diversity of stingless bee by Sawatthum and Kumlert (2015), they have reported Amaranthaceae as an important source for stingless bee since they can thrive in all kinds of environment. Asteraceae has been reported throughout the year by Suryanarayana *et al*. (1990) and Fabaceae has also been found as major source by Biesmeijer *et al*. (1999).

The families of Euphorbiaceae, Acanthaceae, Myrtaceae were also present in either any two of the seasons. Similar findings has been reported by Kajobe

(2006) where he stated the importance of Euphorbiaceae, Acanthaceae and Myrtaceae to the stingless bee.

5.5. FAMILY AND PLANT GROUP DISTRIBUTION OF POLLEN TYPES

The 93 pollen types identified, when considered up to family level, was found to be distributed among a total of 31 families (Table 12). The familywise distribution revealed that the family Fabaceae had highest number of pollen types followed by Asteraceae (Fig. 5). The higher representation of Fabaceae family can be explained by the large amount of pollen offered by flowers over long periods of time (Saravia- Nava, 2018). Similar findings has been reported by Terrab *et al.* (2005), Kajobe (2007) as well as Layek and Karmakar (2018) where maximum pollen types were identified from Fabaceae followed by Asteraceae.

The families Arecaceae, Cucurbitaceae, Myrtaceae and Euphorbiaceae were also well represented in pollen and honey samples indicating their importance and contribution to the bee diet. The same can be observed in the study conducted by Danaraddi (2007) in which he highlighted the occurrence of Arecaceae, Cucurbitaceae and Myrtaceae as important sources of stingless bee. The findings of Bisui *et al*. (2019) also lies well in agreement with higher representation of families Fabaceae, Asteraceae, Euphorbiaceae, Cucurbitaceae and Myrtaceae.

In the present study, 83 plants were identified upto species and genera level which comprised of 10 trees, 14 fruits and plantation crops, 27 aromatic and medicinal plants, 7 vegetables, 8 ornamentals, 16 weed plants and 1 oilseed (Table 13). Similar findings has also been reported by Chaturvedi (1989) who stated that forest trees and associated plants are well represented in honey samples, also the practice of cultivation on deforested lands resulted the increase in influence of fruit crops and entomophilous crops on pollen spectra. The higher number of medicinal and aromatic plants (Fig. 6) might be due to the fact that the tiny size stingless of bees enable them to enter the small flowers of these plants (Jongjitvimol and Wattanachaiyingcharoen, 2006). Vazhacharickal and Jose (2016) in their study has

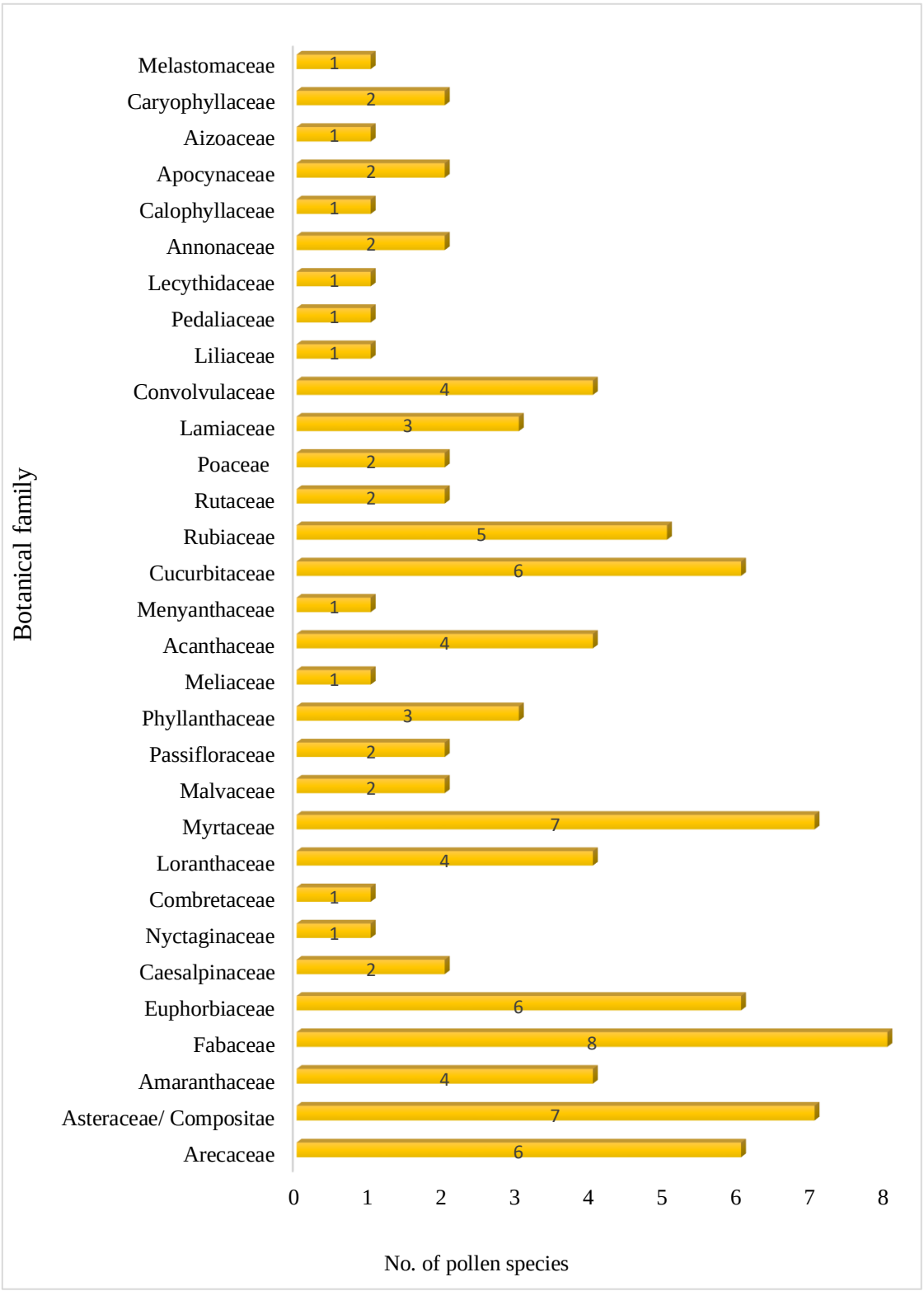


Fig 5. Botanical family representation in identified pollen types

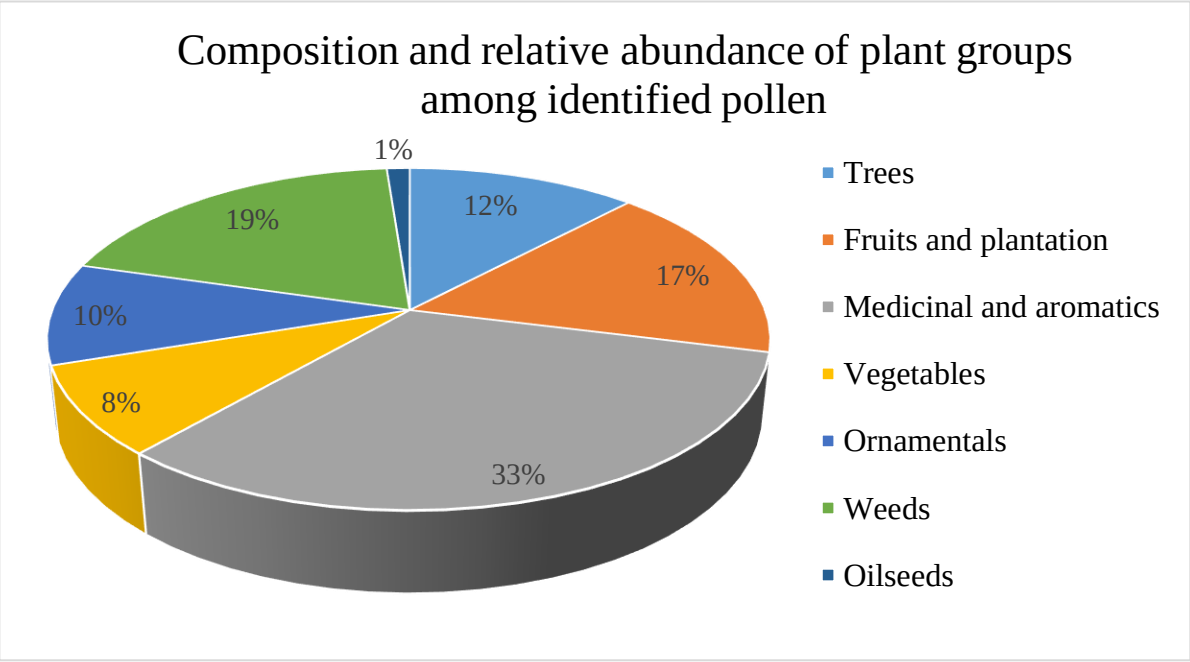


Fig 6. Composition and relative abundance of plant groups among identified pollen

stated that stingless bee prefers ornamentals followed by medicinal plants, fruit crops, trees and vegetables.

5.6. POLLEN METABARCODING

The present study used combination of DNA barcode regions and available database of floral and phenological information to identify pollen to the suitable taxonomic levels. The samples were tested for barcode regions of rbcLa, rbcbL, tnrH, psbA, ITS4, ITS5, matK1 and matK3 as these were promising standard marker primers (Kamo, 2018).

The use of multiple DNA barcode regions is desirable as it creates high differentiating power and accurate results through cross checking (Galimberti *et al*., 2014). The ITS region were successfully used to identify plants in earlier studies (Chen *et al*., 2010; Buchheim *et al*., 2011) whereas tnrL-tnrF combination have proven variable enough to allow identification upto genus level (Kraaijeveld *et al*., 2015). The choice of barcode regions from among standardized region is supple and can be revised according to the level of taxonomic deviations.

The composite sample from different locations in a season was analysed using high throughput sequencing technique *i.e*. next generation sequencing and BLAST search was conducted against NCBI database. A total of 47 plant species were obtained (Table 14) whose presence in sample is predictable and twenty species out of it were highly predictable accounting to their high hits and E- values. High throughput sequencing is found to be extremely useful in taxonomic evaluation of mixed pollen samples obtained from bees as they are finer detailed and comparable in less time and no palynological expertise (Smart *et al*., 2017).

As multiplexed next-generation sequencing aids us to achieve list of species in a mixed sample, it also has shortcomings. The technique has its own limitations as it relies much on the basic reference database and bioinformatical classification algorithm. Except for the ITS2 marker which is the most studied of all, every alternative markers lacks an all-inclusive reference database and taxonomic

classifications are thus based on BLAST search against sequences from a universal database, or locally existing alternate databases or freshly obtained samples itself. BLAST searches are generally based on local alignments which uses only a portion of the sequence for classification and moreover presents difficulty in interpreting the results especially when result shows hits for several dissimilar taxa (Sickel *et al*., 2015). Also, there is less reliability in quantification than other techniques which arise from the variation in plant species and their genetic material, thereby increasing the risk of missing a species from the sample altogether.

Several families that were identified through light microscopy were not detected during metabarcoding as only five species were common to both light microscopy and metabarcoding. Chen *et al*. (2010) reported the inability to classify some of the plant taxa members such as Lamiaceae, Salicaceae and Vitaceae due to the incompleteness of reference database. Also the drawbacks of existing protocols such as less number of samples being processed simultaneously and more number of steps which might result in human errors. Although, the presence of some species unidentified in light microscopy among the barcode detected taxa be due to its low abundance in the samples.

5.7 TOTAL POLLEN DENSITY

In the study, the total pollen density varied between locations within the seasons and also between seasons (Table 15.). The investigation on pollen count of honey samples determined that most samples were from Group II (20,000- 1, 00,000 TNP/mL). The amplitude of estimated pollen concentration of honey samples demonstrated that the minimum and maximum values recorded were similar to those obtained by Ramirez- Arriaga and Martinez- Hernandez (2007) and de Novais and Absy (2015) which ranged between Group I and IV.

The grain size difference of the pollen may be the reason for the difference in pollen densities of honey from various locations. According to Demianowicz (1964), diameter of pollen grain associates with its abundance in honey, hence smaller the grain size, greater its incidence. The greater occurrence of Group II

honey among the locations can be considered normal as stated by Maurizio (1976) that most of the honey falls into this category. There were no honey samples in Group I (low), IV or V (high). The groups with lower pollen grain concentration might be indicating under represented nectar species and vice versa (Moar, 1985).

The pollen density in midlands was higher during the northeast monsoon season and lower comparatively during the dry season whereas lower density was recorded in uplands during the northeast monsoon as compared to the dry season. This variation in pollen density between midlands and highlands during different season suggest that the availability of pollen resources vary in both the lands. Pyke (1984) stated that bee chooses whether to forage at a particular resource based on the continual decision making according to the amount of resource which varies with time and space.

The pollen density in samples was higher in northeast monsoon season than dry season. The importance of pollen in the diet of bees as it is the main source of protein was reported by Kevan and Baker (1983) and Bibi *et al*. (2008). Protein in pollen is essential for the brood development and for young bees, the intake of which is known to improve physiological metabolism, immunity and tolerance to pesticides (Wahl and Ulm, 1983; Alaux *et al*., 2010; Alaux *et al*., 2011; Ament *et al*., 2011).

The difference between the midland locations and upland locations within a season may be due to the difference in topography, temperature and humidity variation prevailing in the regions. The influence of climatic factors on the flower development and pollen production and hence the foraging behaviours of bees were reported by Le Conte and Navajas (2008). Also, the resource plants differ in upland and midland which results in varied availability within the same time frame and hence the foraging by bees. The floral preferences vary which may result in depletion of resources in a particular season in a location which can be also be attributed as a cause for lower pollen density in certain locations. There had been reports in the findings of Foster *et al*. (2014), Russell *et al*. (2016) about bees

showing inclinations to plants providing only pollen resources or plants with an attractive floral display.

5.8. FORAGING ACTIVITY OF STINGLESS BEE

In the study, the activity of *T. travancorica* during northeast monsoon, dry and southwest monsoon season varied at different hours of the day (Table 16-27). Weather and seasonal patterns have had a crucial impact on the foraging behaviour of stingless bees and the number of active bees is affected by temperature and humidity (Bartareau, 1996; Nieh and Roubik, 1998; Oliveira-Abreu *et al*., 2014).

5.8.1 Activity of outgoing foragers

The number of outgoing foragers during southwest monsoon season the highest activity was observed at 1000 to 1100 h which was on par with morning hours until 1200 h. The lowest occurrence of outgoing foraging was at 0600 to 0700 h in all seasons. Azmi *et al*. (2015) reported a peak of outgoing at 1000 hrs in June month and a lower activity level in early morning hours. During the northeast monsoon season was highest at 0800 to 0900 h (Fig. 7) which was significantly different from all other hours whereas in dry season the peak of activity was attained during 0900 to 1000 hrs which again differed considerably from rest of the day. Bharath *et al*. (2020) stated that the outgoing activity became critically high during 0900 to 1000 h in March to May period.

During northeast monsoon season, the month of November had a higher activity of stingless bees which was on par with December (Table 16). April had maximum activity in dry season, which differed significantly from all months, and minimum activity was observed in the month of January (Table 17). This is in **a**ccordance with the observation of the lowest activity in December and January by Vijayan *et al*. (2018) as they are the coldest months. Meanwhile, there was no significant difference between any months and the outgoing was less during southwest monsoon season (Table 18), as was elucidated in the investigations of Jaapar *et al*. (2018) where the foragers remained inside the nest until the rain

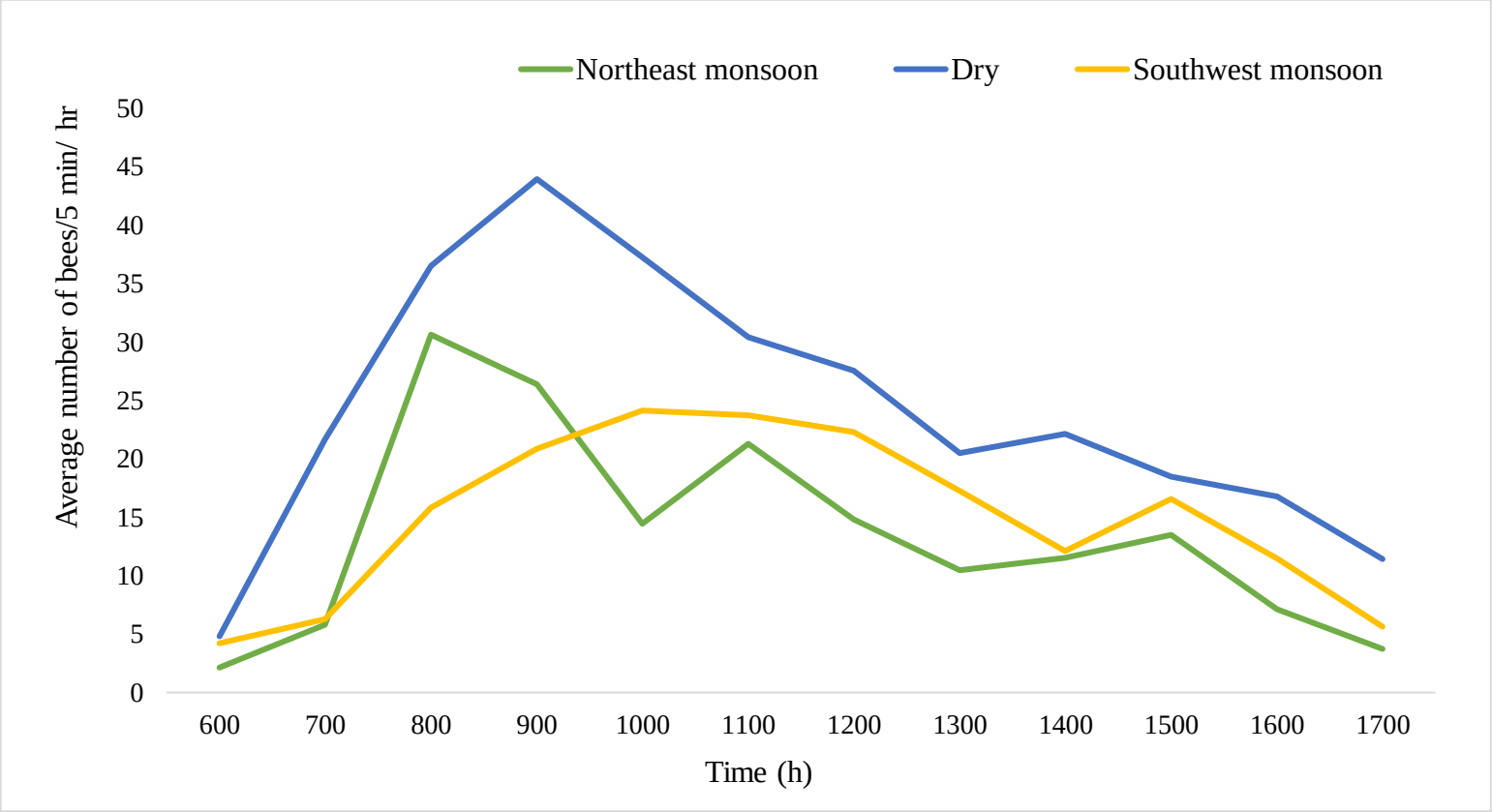


Fig 7. The foraging activity of outgoing foragers during northeast monsoon (November- December), dry (January- May) and southwest monsoon (June- August) season

stopped. The least outgoing behaviour was observed during the northeast monsoon season while highest was during dry season.

5.8.2 Activity of incoming foragers with pollen load

The number of pollen foragers was highest at 0900 to 1000 h which was on par with 0800 to 0900 h during northeast monsoon (Table 19), while lowest activity was recorded in early in the morning (0600 to 0800 h) and late in the evening (1600 to 1800 h). In dry, the least activity was at 0600 to 0700 h and 1800 h, while the peak was attained at 0900 h (Fig. 8) which was on par with 1000 h. This corresponds to the availability of the resource (Roubik, 1989) which also have been observed by de Bruijn and Sommeijer (1997) where the pollen was collected earlier in the day. Peak activity of pollen foragers was observed at 1100 h, on par with other morning hours during southwest monsoon season whereas fewer numbers were observed during early morning and late evening hours. In all three seasons, a slight peak was observed during the afternoon at 1500 h, which can be confirmed from conclusions by Devanesan *et al*. (2002) who also observed the same.

There was no significant difference between both months in northeast monsoon, although higher activity was in November than in December. While in the dry season, the greatest movement was in April which was on par with March and February (Table 20). Least number of foragers was detected in May. Pollen was collected more in the summer than in the spring (Ferreira Junior *et al*., 2010) which may be in line with the observation for higher activity in April. Maximum activity in the southwest monsoon season was recorded in the month of August (Table 21) which was on par with July. High pollen availability during the rainy season is reported (Faria *et al*., 2012) attributed to the influence of rainfall on the abundance of pollen-providing plants, a characteristic of the tropical environment which in turn increased pollen storage (Aleixo *et al*., 2017). Significantly, the lowest activity was observed during the month of June. Keppner and Jarau (2016) also confirmed a reported decrease in pollen collection in *Trigona fuscipennis* Friese at the commencement of rain. The incoming with pollen was least detected

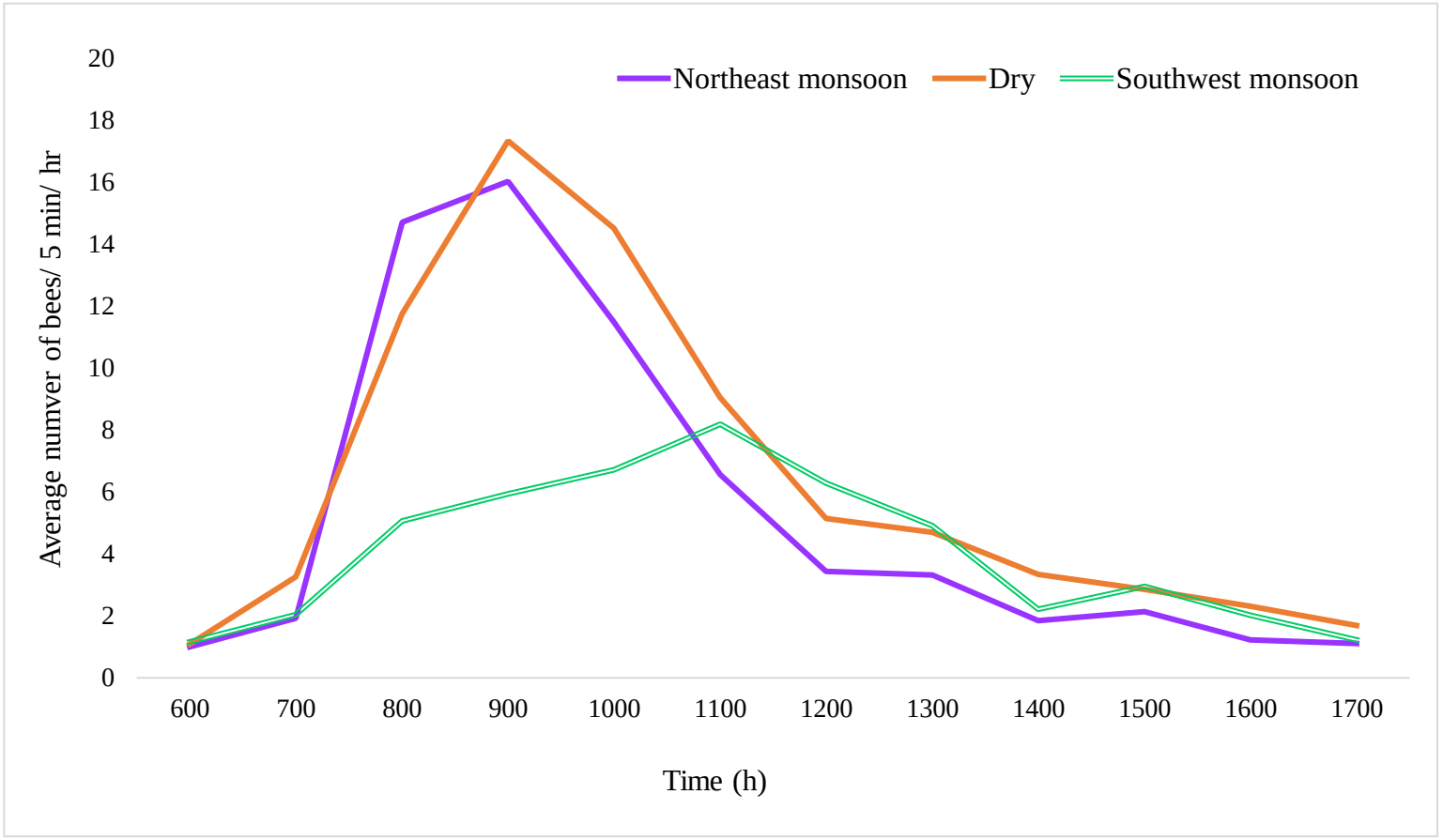


Fig 8. The foraging activity of incoming foragers with pollen during northeast monsoon (November-December), dry (January- May) and southwest monsoon (June- August) season

during southwest monsoon season whereas highest was during northeast monsoon season.

5.8.3 Activity of incoming foragers without pollen load

In northeast monsoon season, the peak of activity was at 1200 h which was on par with 1100 h (Table 22). Whereas in dry season the activity increased abruptly from 0700 hrs to reach a peak at 1000 h (Table 23) which was on par with the rest of the morning hours. Similar findings were also made by Ferreira Junior *et al*. (2010) who stated that nectar collection during winter months is more common during the late morning and early afternoon and during all hours in spring and summer. During southwest monsoon season the activity was highest at 1300 h which was on par with 1000, 1100 and 1200 h and the activity stayed high all throughout the afternoon hours until 1600 h (Table 24). Reports of do Nascimento and Nascimento (2012) also shows that the activity of nectar and other liquid collection showed no noticeable peak during the rainy season. The minimum activity was observed at 0600 h during all seasons.

In northeast monsoon season, where the highest activity was observed during November as compared to December, there was a substantial difference between months. In dry season, in the month of April, the number of incoming foragers without pollen load was maximum, which differed significantly from all other months and the minimum was observed during January. Incoming foragers without pollen load had low activity during the southwest monsoon season (Fig. 9), but there was no significant difference between months and maximum activity was observed during June. According to Danaraddi (2007) and Barbosa *et al*. (2016), this can be justified by the lower activity reported during wettest months marked by high rainfall and strong winds. The incoming foragers without pollen were least observed during northeast monsoon season and highest during dry season. Saufi and Thevan (2015) have also recorded greater collection of liquid resources during the dry season and lowest during the rainy season.

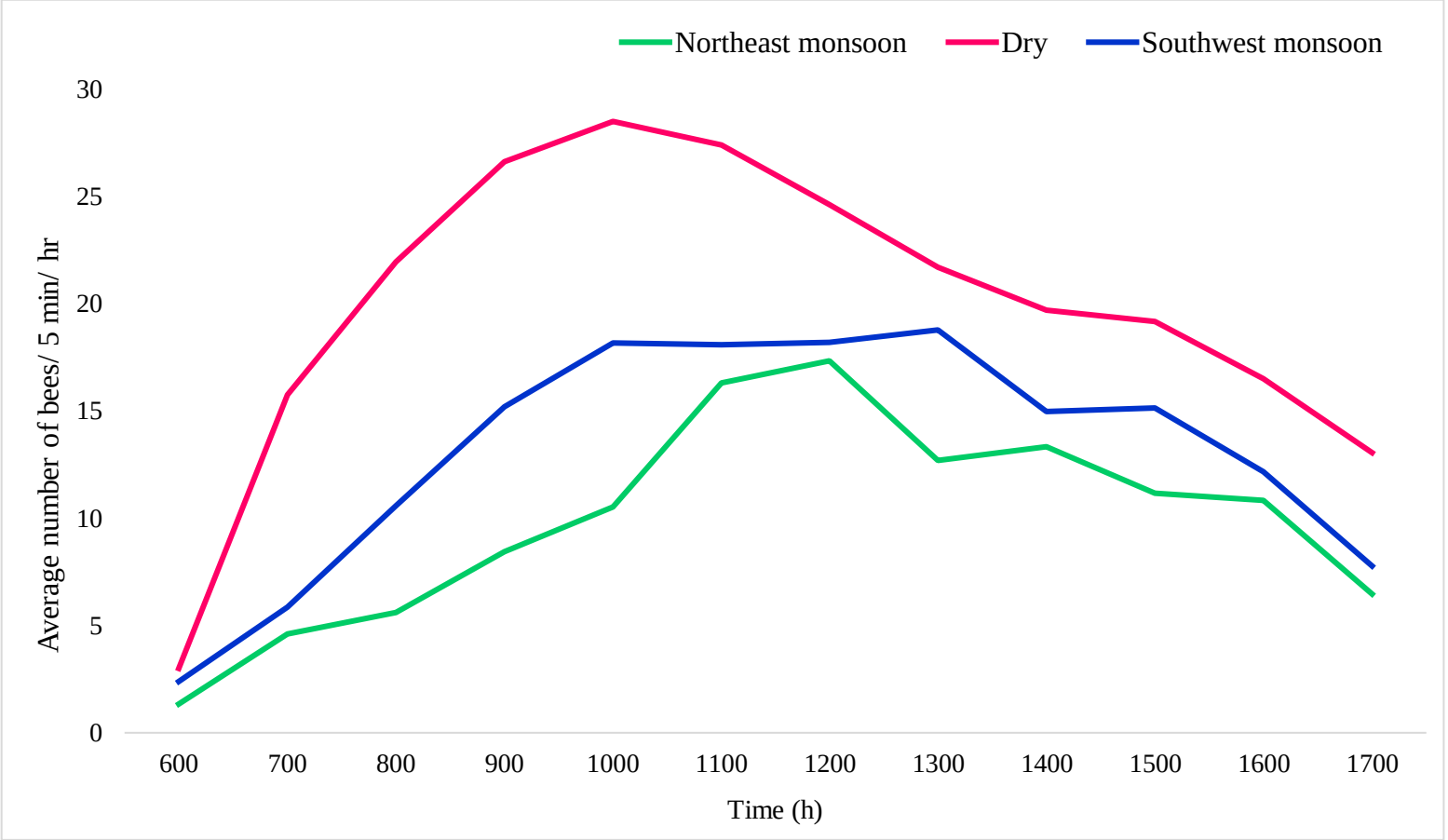


Fig 9. The foraging activity of incoming foragers without pollen during northeast monsoon (November-December), dry (January- May) and southwest monsoon (June- August) season

5.8.4 Activity of foragers regardless of the season

The lowest activity in foraging by both outgoing and incoming was recorded during December and January. Pedro and Camargo (1991) have recorded the bee activity to be the lowest during the coldest months and activity was lowest in the months of December and January in Kerala (Devanesan *et al.*, 2002). The peak activity of outgoing was observed in the month of April (Fig. 10) followed by March and February, the same observed by Managanvi *et al*. (2012) where the highest of all activities was observed from February to May, with peak activity being in April. The maximum activity of incoming foragers with pollen was recorded in the April followed by February and March (Table 25). Meanwhile, the maximum activity of incoming bees without pollen was found in April followed by March and May. Layek and Karmakar (2018) also found outgoing and incoming foragers to be highest during February to April period.

The least activity of outgoing foragers during a day was at 0600 h, then gradually increased in the morning to reach a peak at 0900 h and then decreased until 1800 h in the evening. According to Pereboom and Biesmeijier (2003) the higher activity during morning hours was to fulfil the nutrient requirement and to retain the intranidal temperature at low temperature. During the morning hours, the number of foragers was comparatively greater than in the afternoon hours, which was similar to observations of Patnaik *et al*. (2005) and Prasad and Patnaik (2005).

The incoming bees with pollen load were lowest at early morning hours from 0600 to 0800 h and late evening from 1600 to 1800 h (Table 26). Heard and Hendrikz (1993) concluded from his investigations that the temperature threshold of workers restricted flight activity in the morning and lower radiation level was emerging as a limiting factor in evening hours. Around 0900 h the peak of activity was reached (Fig. 11) and pollen foragers were more active throughout the morning hours until 1100 h, then it declined. This was consistent with the findings of de Bruijn and Sommeijer (1997) and Saravanan and Alagar (2007) in which pollen gathering was mostly active during late morning hours between 0900 and 1100 h.

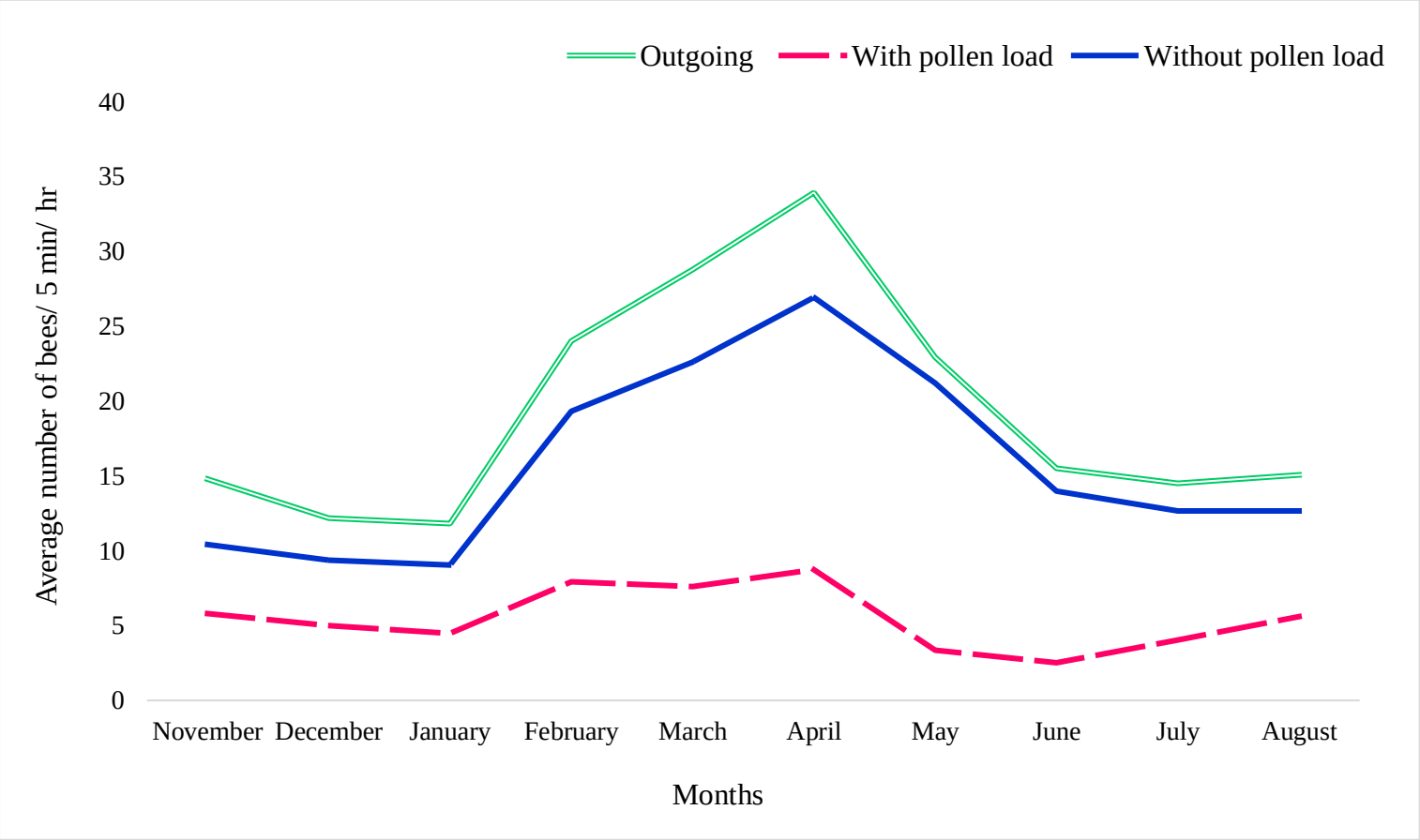


Fig 10. The activity of foragers in different months

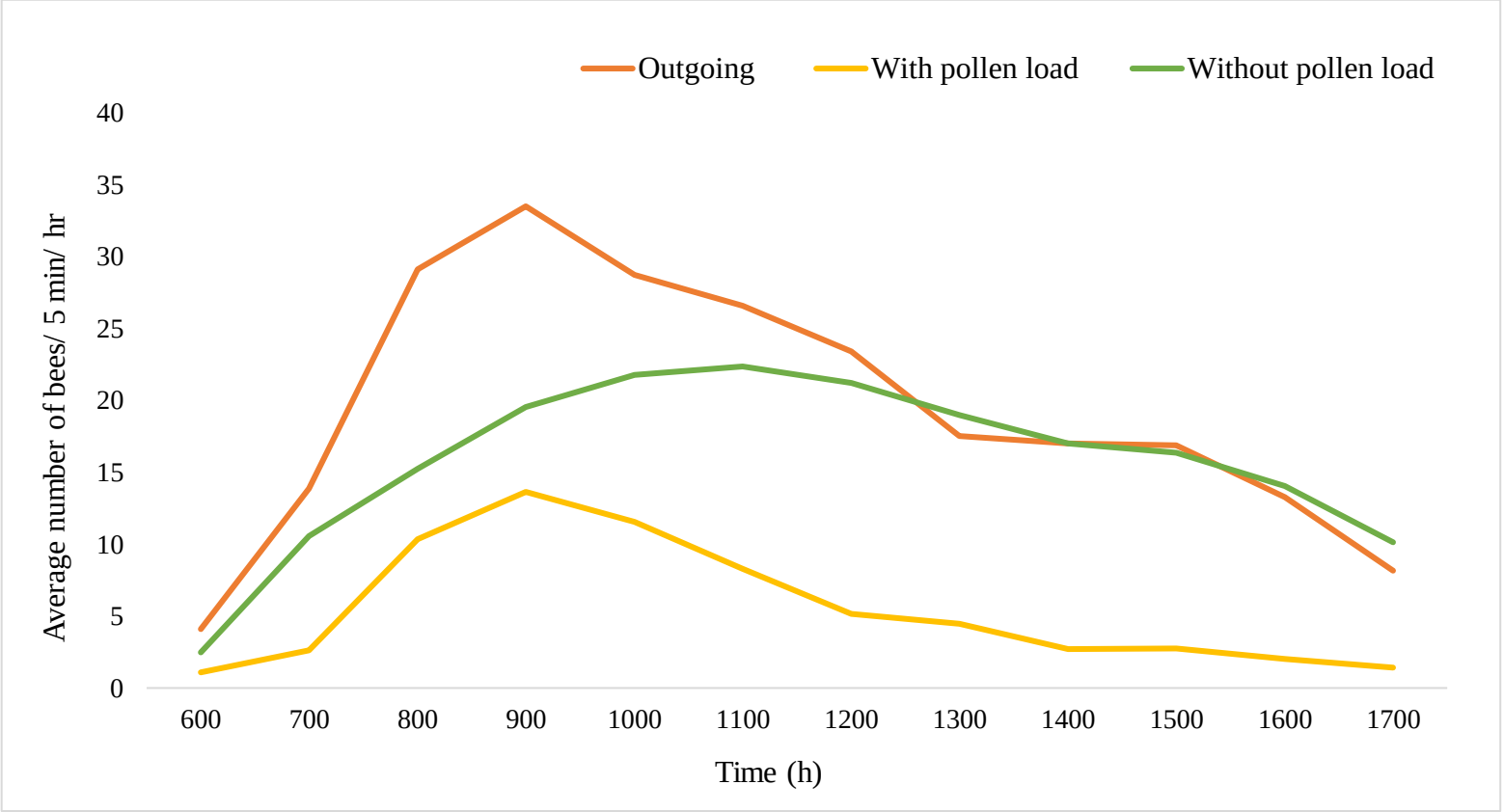


Fig 11. The activity of foragers in different hours

The incoming foragers without pollen load were active all day (Table 27) except for early morning hours. The peak of activity was reached at 1100 h and remained nearly constant until 1300 h after which it gradually decreased. Inoue *et al.*, (1985) reported that the nectar foraging occurred between 0900 to 1400 h in *Heterotrigona itama*.

5.8.5 Foragers with and without pollen load

When a comparison is made between foragers with and without pollen load are done we can see that pollen foraging was more active in the morning hours and foragers without pollen loads were more visible around mid-day and afternoon. Pollen foraging significantly reduced in the afternoon hours which might be due to the depletion in the amount of resource as a result of foraging throughout morning. The profitability of nectar is indicated by sugar concentration and quantity (Real, 1981), and the nectar concentration increases over the day due to evaporation, hence the most profitable time for nectar collection seems to be around the afternoon (Roubik and Buchmann, 1984; Inoue *et al*., 1985). This can be confirmed by studies of Nagamitsu and Inoue (2002) who reported more pollen foragers in morning hours and nectar foragers in afternoon hours.

5.8.6 Relative abundance of incoming foragers with pollen loads

The numbers of pollen foragers in the total incoming foragers was found to be close to 25 per cent which indicates that pollen foragers are less as compared to the non-pollen forager or more accurately incoming foragers with nectar (Table 28). They were found to be greater in number than foragers with pollen load in any hour of the day or for any month. The finding lies in line with the studies of Ferreira Junior *et al*. (2010) and Layek and Karmakar (2018) that the most frequently collected resource was nectar/water followed by pollen.

There are two possible explanations for this. This may imply that collecting pollen load takes more time taken, as much as 50 per cent extra than nectar or liquid foraging. Also, this might be the effect of foragers spending less time per flower

but visiting more number of flowers per flight than nectar foragers (Biesmeijer and Toth, 1998). A remarkable activity of pollen foraging occurred in November and December, pointing towards the response of bees towards an increased colony requirements of pollen during that period. However, Devanesan *et al*. (2002) concluded that pollen foraging activity was lowest during this period, evidently the intimation of changing climate during the span which also emphasize the changing behaviour of bees.

The months of May and June had the lowest of pollen foraging activity clearly due to the monsoon showers during the period. The months following the monsoon showers, July and August had higher activity which was the blooming period for most of the plants due to the influence of rain, identical to the findings of Faria *et al*., 2012 that the months following rain was observed with greater activity of pollen collection and wettest months had lowest activity (Barbosa *et al*., 2016). The pollen foraging was continuous throughout the study period across seasons and higher pollen availability followed with higher foraging (Aleixo *et al*., 2017). The pollen foraging was notable during summer months, which was also in accordance to more pollen foraging during March followed by February as stated by Vijayan (2018).

Summary

6. SUMMARY

The study entitled 'Melissopalynological studies on stingless bee *Tetragonula travancorica* (Apidae: Meliponini) was conducted at Department of Agricultural Entomology, College of Agriculture, Vellayani and in the apiaries of bee farmers in Thiruvananthapuram district during the period 2017-2020.

Sampling was done from eighteen locations of Thiruvananthapuram district, located at southernmost part of Kerala. In the study, fourteen locations were midland and rest four were uplands. Samples of pollen and honey were collected from three parts of hive which was selected randomly on field. The study period was distributed over three season Northeast monsoon season (October- December), Dry season (January- May) and Southwest monsoon season (June- September). The foraging activity studies on hives were conducted in the campus of College of Agriculture, Vellayani from November 2018- August 2019 which was also distinguished into three seasons.

Observations and records of pollen identification in pollen and honey samples, distribution of pollen into frequency classes and frequency of occurrence, seasonal variation in pollen types, total pollen count of honey samples and foraging hive activity were made. The data of the study analysed using statistical techniques were presented in tables and figures and discussed in previous chapters. The salient findings of this study are summarised below.

- A total of 115 pollen types were obtained over the seasons out of which ninety three were identified. Sixty seven were identified up to species level, sixteen up to genera level and ten up to family level. Twenty two were unidentified pollen types.

- The important pollen types identified were *Cocos nucifera, Mimosa pudica, Mimosa diplotricha, Milletia pinnata, Macaranga peltata, Alternanthera sessilis, Peltophorum pterocarpum, Sweitenia mahagoni, Tridax procumbens, Mikania micrantha, Amaranthus spinosus, Amaranthus viridis, Borassus*

flabellifer, Ageratum conyzoides, Ceiba pentandra, Passiflora edulis, Caesalpinia echinata, Boerrhavia diffusa, Amaranthus hybridus, Delonix regia, Terminalia arjuna, Psidium guajava, Psidium araca, Momordica charantia, and *Syzygium cumini.* The important species identified up to genus level were *Areca sp., Eucalyptus* sp., *Glochidion* sp.1, *Glochidion* sp.2 and *Manihot* sp.

- The most predominant species in all seasons were *C. nucifera, M. pudica* and *M. pinnata*. The secondary pollen types observed were, *M. peltata, T. procumbens, S. mahagoni, P. pterocarpum, A. spinosus, A. sessilis* and *Areca* sp.

- Total pollen count per mL of honey, when considered revealed that honey samples in both the season falls under either Group II (20,000-1, 00,000 pollen grains/ mL) or Group III (1, 00,000-5, 00,000 pollen grains/ mL). The total pollen count of honey sample ranged from 27,000 TNP/mL (Group II) to 2, 94,000 TNP/mL (Group III).

- Pollen density was varying across locations during each season, in each location during different seasons and also between elevations of land. The pollen count in midlands was lower during dry season while during northeast monsoon season the pollen count in uplands was lowered radically.

- The maximum pollen density in honey samples during both the northwest monsoon and the dry season was in midland (2, 08,000 pollen grains/ mL and 2, 94,000 pollen grains/ mL). The highest density of honey from upland during both the seasons were 1, 83,000 pollen grains/ mL and 2, 52,000 pollen grains/ mL, respectively. The lowest pollen density was recorded from midland location in both the seasons.

- The location with the maximum number of pollen count was Panacode (2, 94, 000 pollen grains/ mL) which is a midland while lowest count was observed from Aruvippuram (27, 000 pollen grains/ mL) which also comes under midlands.

- Frequency distribution of pollen in honey samples revealed that *C. nucifera* and *M. pinnata* occurred in more than 50 per cent of the total locations, hence very frequent. Whereas the frequency distribution of pollen in pollen loads showed that *C. nucifera* and *M. pudica* frequently occurred in more than half of locations.

- The maximum seasonal variation in pollen types was observed during dry season (78 no.s) followed by northeast monsoon season (66 no.s) and southwest season (62 no.s).

- Maximum pollen types occurred in family Fabaceae (8 no.s) followed by Asteraceae and Myrtaceae (7 no.s each). Medicinal and aromatics (27) dominated the identified species followed by weeds (16 no.s), fruits and plantation (14 no.s), trees (10 no.s), ornamentals (8 no.s), vegetables (7 no.s) and oilseeds (1 no).

- Metabarcoding of pot pollen revealed the predictability of presence of twenty plant species in the collected samples of pollen loads out of the forty seven species listed out, considering its high hits for sequence reads. Plant species like *Amaranthus tuberculatus*, *A. spinosus*, *A. caudatus*, *A. hybridus*, *A. hypochondriacus*, *A. palmeri*, *Malvales* sp., *Benincasa hispida*, *Lagenaria siceraria* and *Muntingia calabura* are included in the twenty species.

- Foraging studies revealed that the hive activity was highest during dry season than other two seasons and reached a peak during hotter months April, February and March. The least activity was observed during colder months December and January.

- The outgoing foragers and incoming foragers with pollen were most active during 0800-1100 hrs while incoming foragers without pollen loads were active around 1000-1300 hrs. The least activity of a day was found from 0600-0700

hrs and 1700-1800 hrs. The activity gains momentum by 0800 hrs and peak activities were observed until afternoon hour 1500 hrs.

- The highest activity of outgoing foragers in northeast monsoon was observed during November, during April in dry season and in June during south-west monsoon season. Although the incoming foragers with pollen was most active in November during north-east monsoon, April in dry season and August in south-west monsoon season. The foragers incoming without pollen were maximum during November in north-east monsoon season, April in dry season and June during south-west monsoon season.

- The incoming foragers with pollen was most frequently encountered in morning hours till mid-day while nectar foragers were more active around mid-day and few hours into afternoon. The number of pollen foragers were less than the foragers without pollen. The highest activity of pollen foraging was observed in November and December which was in northeast monsoon season while lowest was observed during May and June coming under southwest monsoon season.

6.1 Future line of work

1. Documentation of genome sequences of more floral resources with a molecular level approach have to be undertaken.
2. Comprehensive documentation of floral resources of stingless bees have to be undertaken in whole Kerala through more field studies.
3. Floral calendar have to be prepared in order to help farmers practicing meliponiculture.
4. Studies on change in foraging behaviour during the lifespan of stingless bee have to be undertaken by developing techniques for marking individual bees.

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Abstract

MELISSOPALYNOLOGICAL STUCIES ON STINGLESS BEE *Tetragonula travancorica* (APIDAE: MELIPONINI)

by

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ABSTRACT

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ABSTRACT

The present investigation entitled "Melissopalynological studies on stingless bee *Tetragonula travancorica* (Apidae: Meliponini)" was conducted at the Department of Agricultural Entomology, College of Agriculture, Vellayani, during 2017-2020. The objective was to study the foraging activity of stingless bee *T. travancorica* Shanas and Faseeh and to identify its floral pollen resources occurring in Thiruvananthapuram district. Eighteen locations were selected across the district on the basis of land elevation and composite sampling of pollen and honey was done purposively from hive during the three seasons, *viz.* Northeast monsoon season (October- December), Dry season (January- May) and Southwest monsoon season (June- September). The collected samples were processed and analysed using standard acetolysis procedure recommended by the International Commission for Bee Botany (Louveaux *et al*., 1978). The pollen load in honey samples were determined and pot pollen collected during the period was subjected to metabarcoding. Foraging activity of stingless bees in the hive was studied and observations were recorded fortnightly from 0600 hrs to 1800 hrs during all the three seasons from November to August.

In the study, out of 115 plant types recorded as foraging sources, 93 were identified. Sixty-seven plants were identified up to the species level, 16 to genus level and 10 up to family level. Important species identified were *Cocos nucifera, Mimosa pudica, Mimosa diplotricha, Milletia pinnata, Macaranga peltata, Alternanthera sessilis, Areca sp., Peltophorum pterocarpum, Sweitenia mahagoni, Tridax procumbens, Mikania micrantha, Amaranthus spinosus, Amaranthus viridis, Borassus flabellifer, Ageratum conyzoides, Ceiba pentandra, Passiflora edulis, Caesalpinia echinata, Boerrhavia diffusa, Amaranthus hybridus, Delonix regia, Terminalia arjuna, Psidium guajava, Psidium araca, Momordica charantia, Eucalyptus* sp., *Glochidion* sp. and *Syzygium cumini*. The most predominant were pollen of *Cocos nucifera*, *Mimosa pudica* and *Milletia pinnata* during all seasons. The secondary pollen types observed were *Macaranga peltata, Tridax procumbens*, *Sweitenia mahagoni*, *Peltophorum pterocarpum*, *Amaranthus spinosus*,

Alternanthera sessilis and *Areca* sp. Most frequently observed pollen type in honey samples were *C. nucifera* and *M. pinnata* whereas *C. nucifera* and *M. pudica* were very frequent in pollen samples common to all seasons. The botanical family which had the highest recorded pollen type was Fabaceae (8) followed by Asteraceae (7) and Myrtaceae (7). Metabarcoding of pollen revealed that *Amaranthus tuberculatus*, *A. spinosus*, *A. caudatus*, *A. hybridus*, *A. hypochondriacus*, *A. palmeri*, *Malvales* sp., *Benincasa hispida*, *Lagenaria siceraria* and *Muntingia calabura* were the predictable candidates as observed by their bit scores and E-value. The total pollen count of honey samples ranged from 27,000 TNP/mL (Group II) to 2, 94,000 TNP/mL (Group III). A marked difference was observed between mid-land and upland and also among locations during both the seasons. The pollen count in midlands was lower during dry season whereas during northeast monsoon season the pollen count in uplands was radically lowered.

Foraging activity of stingless bees revealed that the highest activity occurred during March and April (hotter months) while the lowest during December and January (colder months). The maximum number of outgoing and incoming foragers with pollen was recorded during April, March and February whereas, incoming foragers without pollen was highest during March, April and May. The highest average of outgoing foragers and incoming foragers with pollen load was observed during 0900 to 1200 hrs while, in incoming foragers without pollen load, it was from 1000 to 1300 hrs. The ratio of pollen foragers to total incoming foragers was highest during November followed by December and the least during May and June.

Thus, melissopalynological studies revealed the presence of 115 foraging sources for *T. travancorica* in Thiruvananthapuram district while foraging studies revealed maximum foraging activity during hot dry season. The study highlights that the stingless bees exploit diverse floral resources available in their surroundings.