POLLINATION ECOLOGY OF SOLITARY POLLEN BEES

By

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(2017-21-035)

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THESIS

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DECLARATION

I hereby declare that the thesis entitled "**Pollination ecology of solitary pollen bees**" 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 to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Date: 24.04.2023 **(2017-21-035)**

CERTIFICATE

Certified that this thesis entitled **"Pollination ecology of solitary pollen bees"** is a bonafide record of research work done independently by **Mrs. Anusree Padmanabhan P. S. (2017-21-035)** 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 her.

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Introduction

1. INTRODUCTION

Biodiversity is the foundation of any ecosystem service to which human well-being is directly linked. Crop pollination is a keystone process in maintaining biodiversity and is considered an endangered ecosystem service that has attracted serious concern in recent years as it plays a critical role in global biodiversity (Steffan-Dewenter *et al*., 2005). Among the crop pollination services, animal pollination is vital for the sexual reproduction of many crops and a decline in the pollinating species leads to a parallel decline of the plant species (Biesmeijer *et al*., 2006).

Approximately, 80 per cent of all angiosperms are pollinated by animals, including vertebrates and mammals, but the major pollinators are insects (Rehel *et al*., 2009; Hallmann *et al*., 2017). Thus, entomophily is by far recognized as the most common mean of pollen transfer in plants, and it played a vital role in the evolution of angiosperms (Cox, 1991; Labandeira *et al*., 1994; Soltis *et al*., 2019). According to The Economics of Ecosystems and Biodiversity (TEEB, 2010) the total economic value of insect pollination was estimated to be € 153 Billion, which equates to 9.5 per cent of agricultural production.

Among the pollinating insects, honeybees have often been credited with the title of most efficient pollinator with excellent pollination services to crop plants that were performed by other bee species (Abrol and Shankar, 2014). More than hundreds of entomophilous crops that were believed to be pollinated with honeybees were later reported to get pollinated with non-*Apis* bees also called wild bees (Parker *et al*., 1987). Comparative studies on pollination services by honeybees and non-*Apis* bees to thousands of angiosperm species surprisingly revealed the significance of non-*Apis* bees in pollination being mistakenly given to honeybees (Nabhan and Buchmann, 1997; Goulson, 2003).

About 70 per cent of the more than 20,000 non-*Apis* bee species in the world are solitary bees (Sgolastra *et al*., 2019). Solitary bees are not only the keystone members of natural ecosystems but also can provide valuable pollination services in agricultural ecosystems (Woodcock *et al*., 2013; Burkle *et al*., 2013). Solitary bees have often proved to be excellent pollinators than honey bees and bumble bees, owing to their morphology and their relatively greater interaction with flower stigmas

(Vicens and Bosch, 2000; Fontaine *et al*., 2006). The pollination services provided by solitary bees are critical to the vitality of ecosystems and agricultural systems alike. In recent years, the research on the significance of solitary bees among non-*Apis* bees has opened a new era in pollinator studies (Sihag, 1988; Batra, 1995; Devy and Davidar, 2006; Pannure, 2016; Bhalchandra and Bhaviskar, 2017; Udayakumar and Shivalingaswamy, 2019; Bhatta and Kumar, 2020; Shivalingaswamy *et al*., 2020; Vijayakumar *et al*., 2022). Bee pollination provides benefits to society by improving their livelihoods and conserving biodiversity by adding to the productivity of agricultural and natural ecosystems (Blitzer *et al*., 2016).

In India, bees are generally known to the public as honey bees and many people are unaware of the existence of solitary pollen bees. Though honey bees constitute less than 0.01 per cent of the total bees worldwide, they have been extensively utilized for the managed commercial pollination activities to major agricultural crops, and are considered the most economically valuable pollinators because of the monetary benefits they provided in the form of honey and wax. Although the significance of honey bees being undoubtedly established as the most efficient pollinator in a developing country like India cannot be ignored, it would be inappropriate when this is being done without ascertaining whether these honey bee species are the exact efficient pollinators of the related flora.

Total negligence of the existence of solitary pollen bees and blind usage of honey bee hives in agricultural lands will not only reduce the effectiveness of crop pollination but also will have a negative impact on honey bee populations. In recent years, the world has faced drastic declines in managed honey bee populations due to several reasons *viz*., climate change, diseases and parasitic mites (Reddy *et al*., 2012; Roy *et al*., 2018). This has raised alertness in various parts of the world as it led to major pollination shortfalls in several crops (Kevan and Phillips, 2001; Garibaldi *et al*., 2009; Cunningham *et al*., 2016). Many countries have started giving awareness of the potential risk of sole reliance on honey bees for agricultural pollination (Klein, *et al*., 2007; Neov *et al*., 2019). This has given insight into the significance of alternate pollinators and their conservation. Moreover, the burgeoning human population and habitat fragmentations are inflicting negative impacts on native solitary bee diversity. The current trend of wavering climate along with these factors will exacerbate

existing pressures on native bee biodiversity and will impose a spiraling effect on already deteriorating ecosystem services (Hegland, 2009).

In Kerala, there have been very few attempts to document and assess the native solitary bee diversity and its conservation. Studies on the effectiveness of solitary bees as pollinators of agricultural and horticultural crops and the impact of pesticide exposure to the visit of solitary pollen bees in various crop ecosystems are scanty. In light of these limitations, this study was taken up as a pilot study in documenting major native solitary bee fauna of selected cucurbitaceous ecosystems in central districts of Kerala. The specific objectives of the study are as follows.

- To document the diversity of all pollinators in selected cucurbitaceous crops and the morphological characterization and barcoding of solitary pollen bees.
- Determining the peak foraging time of solitary pollen bees in selected cucurbitaceous crops.
- Studying the nesting preferences of native solitary bees by providing artificial nesting sites.
- Palynological studies on solitary pollen bees.
- Assessment of the effect of plant protection measures on solitary pollen bee visits to selected cucurbitaceous crops.

Review of Literature

2. REVIEW OF LITERATURE

The literature pertaining to the present study on the "pollination ecology of solitary pollen bees" has been reviewed in the present chapter. The available literature has been classified and presented under specified headings such as insect pollination and its significance in horticultural crops, the importance of insect pollination in cucurbitaceous vegetables, diversity of pollinators in cucurbit ecosystems, determination of pollinator performance, nesting preferences of solitary pollen bees, natural enemies of solitary pollen bees, palynology and effects of plant protection measures on the pollination. Since the available literature on the significance of solitary bees on pollination in selected cucurbit crops is scanty, the review on the subject of pollination has been presented in general, covering the various aspects of the pollination ecology of solitary pollen bees.

2.1 Insect pollination and its significance in horticultural crops

Pollination is a keystone process in both managed and natural ecosystems. The significance of pollinators to global agricultural stability was well established (Stebbins, 1970). From an evolutionary point of view, important pollinators were more likely to be stronger agents of natural selection on floral traits (Heinrich, 1975). Kiester *et al*. (1984) suggested that the co-evolution of flowering plants and their pollinators started about 225 million years ago. Clark and Christie (1988) proved pollination is a key to bio-resource mobilisation to fulfill the need of a rising human population, which aids in increasing the productivity of agroecosystems and other natural ecosystems. Robinson *et al*. (1989) quantified the incidental pollination gains from the existing stock of honey bee colonies at ₹ 1470 crores. However such estimates were considered to be deceptive (Chaudhary and Taori, 1993). Ingram *et al*. (1996) suggested that diversity among species including agricultural crops depended on animal pollination. Thus the pollinators were established as essential agents for diet diversity, biodiversity and the maintenance of natural resources.

Many adult insects visit flowers for food (Proctor *et al*., 1996). Most visitors drink nectar, whose sugars often fuel their immediate energetic needs, particularly for flight. The majority of insect pollinators belong to three orders *viz*., Hymenoptera, Lepidoptera and Diptera (Chaudhary, 1998). Daily (1998) provided subjective

estimates of economic benefits from insect pollinators of 12 entomophilous crops at ₹ 2997 crores annually. Pollination was affirmed to be essential for human food and animal feed resources (Richards, 2001). Stone carvings and bricks from the palace of Assyrian kings depicted the significance of pollen and pollination of fruits such that, pollination enhances the quality and yield of seeds and fruits (Pratap, 2001; Kremen *et al*., 2002). Moreover, their population and diversity serve as bio-indicators of their respective environment (Kremen *et al*., 2002; Klein *et al*., 2003; Tylianakis *et al*., 2004). There is a growing consensus that biodiversity enhances ecosystem function in general (Hooper *et al*., 2005) and the delivery of the ecosystem service of pollination in particular (Nagar and Chaudhary, 2006). This vital ecological function could be performed by a variety of animals, predominantly insects (Klein *et al*., 2007; Kremen *et al.,* 2007). Up to 75 per cent of the crops used for human food require insect pollination (Klein *et al*., 2007). An estimated 35 per cent of crop production, including many of our most nutritious foods, benefit from insect pollination worldwide (Aizen *et al*., 2008; Hoehn *et al*., 2008).

Insect pollination was established as a crucial ecosystem service that was vital for the sexual reproduction of wild plant species (Gallai *et al*., 2009; Ollerton *et al*., 2011) and also for the fruit set of many crop species (Thakur, 2012). Jauker *et al*. (2012) suggested that semi-natural habitats were most important for buffering against temporary dynamic disservices that pollinators face in modern agroecosystems.

In India, about 80 per cent or more of the crop plants were dependent on insect pollination (Garibaldi *et al*., 2013). Insect pollination was worth an estimated 153 billion dollars to global agricultural productivity, accounting for 9.5 percent of global agricultural produce utilised for human sustenance (Garibaldi *et al*., 2013; Kennedy *et al*., 2013). Pollinators greatly enhanced the quality of fruits, nuts, vegetables and oilseeds, etc. thus economic value of crop production was immensely increased (Garratt *et al*., 2014; Klatt *et al*., 2014). Giannini *et al*. (2015) suggested that wild insects were an important but underappreciated group of crop pollinators, representing half of all pollinator visits to crop flowers in agricultural systems worldwide. Chaudhary and Chand (2017) opined that the real benefits of animal pollination were impossible to estimate due to many complexities and it was even harder to quantify the other benefits like ecosystem services as they were more qualitative.

2.1.1 Bees as a major pollinator of crops

Butler and Simpson (1954) estimated that there were about 20,000 bees worldwide. These included about 250 genera, 9 families and, 49 subfamilies (Linsley, 1958). The challenge faced in using non-*Apis* bees as managed pollinators was rather quantity than quality and also management techniques that were developed only for a small number of taxa (Bohart, 1972; Batra, 1977). The role of solitary bees / non-Apis bees / native bees / wild bees as crop pollinators might be substantial in natural ecosystems (Parker *et al*., 1987; Kevan, 1990; Torchio, 1990a). Prescott-Allen and Prescott-Allen (1990) found that approximately 73 per cent of the world's cultivated crops such as cashews, squash, mangoes, cocoa, cranberries and blueberries were pollinated by various bee species, 19 per cent by flies, 6.5 per cent by bats, 5 per cent by wasps, 5 per cent by beetles, 4 per cent by birds and 4 per cent by butterflies and moths. Chagnon *et al.* (1993) found that when present with honey bees, native bees could enhance the effectiveness of pollination. Ironically, it has been recognized that honey bees were the major pollinators in commercial crop production in terms of their pollinating efficiency (Free, 1993; Batra, 1995). Among the hymenopteran pollinator fauna, bees have been considered a priority group as a perfect example of a 'positive externality' in economic parlance (Batra, 1995). According to Richards (1996), bees could be classified into two *i.e*., *Apis* bees and non-*Apis* bees, in that *Apis* bees were widely managed in hives for crop pollination and considered as the most efficient pollinators worldwide.

Nabhan and Buchman (1997) reported that of hundred or so animal-pollinated crops which make up most of the world's food supply, 15 per cent were pollinated by domestic bees, while 80 per cent were pollinated by wild bee species and other wild animal pollinators. Engel and Schultz (1997) reported that among the 20,000 species of bees existing in the world, only eight species of honey bees were recognized with a total of 43 subspecies *viz*., *Apis cerana* (Eastern honey bee), *Apis dorsata* (Giant rock honey bee), *Apis andreniformis* (Black dwarf honey bee), *Apis florea* (Red dwarf honey bee), *Apis koschevnikovi* (Koschevnikov's honey bee), *Apis laboriosa* (Himalayan giant honey bee), *Apis mellifera* (Western honey bee) and, *Apis nigocincta* (Philippine honey bee). Apart from honey bees / *Apis* bees which are eusocial, 85 per cent of bee species in the world were proved to be solitary. Bees evolved from sphecoid wasps during the cretaceous period but they were dissimilar from sphecoid

wasps as they strictly followed a nectar-pollen diet (Heard, 1999). Even though they were morphologically similar to sphecoid wasps, they were easily distinguishable by their branched or plumose hairs, basitarsi of hindlegs, which were wider than other tarsal segments, and lacked strigilus (Michener, 2000). Bees were reported to occur in a wide range of biogeographical regions and habitats (Michener, 2000). This distribution pattern of bees and their species diversity were thought to be linked with host breadth.

Kremen *et al.* (2002) reported that among the 100 crop plants that provide 90 per cent of food supplies to 146 countries, 71 were bee-pollinated (mainly by wild bees) and numerous other plants were pollinated by thrips, wasps, flies, beetles, moths and other insects. The non-*Apis* were proved to be equally effective or better pollinators than *Apis* bees in many crops (Kremen *et al*., 2002; Winfree *et al*., 2007). He also concluded that wild bees were sufficient to provide pollination services for several crops, including for those that were not serviced by honey bees. Michener (2007a) reported that more than 20,000 bee fauna were recorded worldwide, their size ranged from 2mm (1/12 inch) to more than 25 mm (1 inch); exhibited a wide variety of foraging and nesting strategies and varied from solitary to highly social and other life histories (Michener, 2007b). Minckley (2008) opined that areas with the greatest species richness of bees possessed a greater proportion of oligolectic species and fewer social species.

2.1.2 Major challenges in beekeeping

According to Torchio (1990b), modern beekeeping has been facing a slew of problems including honey bee diseases, parasitic mites, the inability of honey bees to work at low temperatures and various other adverse climatic conditions. These declines were rapid over years because of several factors such as intensive agricultural practices (Bjorklund *et al*., 1999), use of pesticides (Kevan, 1999), habitat fragmentation (Kremen *et al*., 2002), climate change (Cane *et al*., 2006), urbanization (Hegland *et al*., 2009), anthropological interventions to natural nesting sites (Kearns and Oliveras, 2009), microwave radiations from mobile towers (Sharma and Kumar, 2010) and competition from non-native species ((Thakur, 2012), *etc.* Beekeepers, policymakers and growers of insect-pollinated plants were more concerned over the recent widespread decline (Colony Collapse Disorder) in honey bee populations. Long

after the remark of the great physicist Albert Einstein on bees, the world started facing the serious threat of losing bees (Pannure, 2016). Many scientific reports pointed out that most pollinator populations have declined to a level that could not sustain their pollination services in agroecosystems or natural ecosystems. The conservation of bee pollinators *i.e*., both *Apis* and non-*Apis* bees has become a major issue of concern because a decline in bee population could seriously affect world food security due to a lack of pollination services (USDA, 2017).

2.1.3 Significance of alternative pollinator fauna

According to Kendall and Solomon (1973), discounting the poorly transferable corbicular pollen pellets in honey bees, andrenid bees carried more apple pollen on their bodies and deposited twice as much pollen per visit. Batra (1977) reported that bees were a tremendously diverse group of insects, where they could be divided into taxonomic groups based on phylogenetic relationships or ecological groups based on natural history attributes such as floral associations, natural habits and social structure. Non- *Apis* bees were often specialized for pollinating on particular flower taxa such as squash, berries, legumes, or orchard crops (Tepedino, 1981). Schemske and Horvitz (1984) reported that differences in morphology, sensory physiology and foraging behavior of insects might result in their effectiveness as pollinators and generalized pollination systems, the pollinator effectiveness often varied among different insect visitors. Heard and Dollin (1998) opined that though stingless bees were generalist foragers, individual colonies or populations demonstrated a tendency to visit particular types of flowers or exhibited a temporary fidelity to specific plant species.

Canto-Aguilar and Parra-Tabla (2000) mentioned the necessity of conservation of non-*Apis* bee populations in agricultural ecosystems over the *Apis* bee populations, considering that the introduction of *Apis* bee populations in natural ecosystems was unnecessary and even detrimental to non-*Apis* bee populations. They have also suggested that the current pandemic of varroatosis among honeybees highlighted the need to find alternative species as managed crop pollinators. Most bumble bees were reported to be generalist foragers, visiting a wide variety of flowers (Kearns and Thomson, 2001). They gather pollen by buzzing flowers, *i.e*., holding the flowers tightly and vibrating their flight muscles with an audible buzz, causing the poricidal anthers to release their pollen (Javorek *et al*., 2002).

The eusocial bees include all species of the genus *Apis*, approximately 400 stingless bees of the tribe Meliponini and bumble bees of non-*Apis* social bee species (Goulson, 2003). Furthermore, several scientific reports highlighted the significance of diverse pollinator guilds for optimal pollination in agricultural landscapes (Kremen *et al*., 2002; Klein *et al*., 2003; Njoroge *et al*., 2004; Kremen, 2005; Biesmeijer *et al*., 2006).

Evaluation of the pollination efficiency of non-*Apis* bee pollinators was one of the first steps in planning successful strategies for their conservation. Sung *et al*. (2006) reported that solitary bees spent a long time on a flower to pollinate which was three times more than honey bees. However, studies which quantified the relationship between crop production and pollinator species richness and functional group diversity were quite rare to establish this fact (Winfree *et al*., 2007; Campos *et al*., 2008; Hoehn *et al*., 2008). Social bees were observed to live as a colony in a nest with one queen and only a few non-*Apis* bee species were reported to live with highly social (eusocial) behavior (Cane, 2008). Some species of bees use plant products such as leaves, plant hairs, oils, resins and fragrances to feed their larvae, build and protect nests, and attract their mates (Abbott *et al*., 2008). The specificity in pollinator fauna was usually associated with more efficient pollination on an individual bee visit basis, which could result in larger and high-quality fruits or seeds from crops (Brunet and Stewart, 2010; Isaacs and Kirk, 2010). Bumble bees were considered important pollinators of crops in temperate regions with poricidal anthers to release such as blueberries, cranberries, and solanaceous plants including tomatoes and eggplants, peppers, strawberries and other *Vaccinium* spp. (Dafni *et al*., 2010).

Bees use nectar as a carbohydrate source and pollen as a source of protein, fatty acids, minerals and vitamins (Ascher and Pickering, 2011; Ollerton, *et al*., 2011). Watson *et al*. (2011) observed that wild bee species richness and abundance were important predictors of seed set in apples whereas, greater abundances of honey bees did not lead to an increase in the number of seeds per fruit. Bees were reported to exhibit a wide range of social behaviours, but they could be classified into two broad categories based on their interdependency, *i.e*., social or solitary (Vaughan *et al*., 2014) and it also proved that there was a growing emphasis on the role of unmanaged or wild bees in agroecosystems among the agriculture and conservation strategies

across the world. According to Martins *et al*. (2015), wild bees had functional traits that were distinct from, and complementary to those of honey bees. The availability of wild bee pollinators in orchards depended on forest and meadow habitats in the surrounding landscape that provided foraging and nesting resources before and after the apple's blooming period (Mallinger and Gratton, 2015). Blitzer *et al*. (2016) along with studies from other crops around the globe, suggested that increasing populations of honey bees would not compensate for the losses of wild pollinators.

2.1.4 Solitary pollen bees and their role in pollination

Daly (1988) opined that, although most non-*Apis* bees were solitary in nesting, the females could be very gregarious, preferring to make their nest side-by-side, in the same area, much like urban apartment dwellers. Such solitary bees with gregarious behavior were the best to manage to get numerous bees all together in a limited space, which could come in handy to manipulate crop pollination. Seed sets significantly increased with increasing numbers of solitary bee species as well as with increasing solitary bee abundance (Torchio, 1990a). Solitary pollen bees could be observed in all habitats, except underwater and in Antarctica (Batra, 1994). Solitary bees evolved from predaceous ancestors such as the solitary mud-dauber wasps in about the middle of the cretaceous period (100 million years ago) when flowering plants became the dominant vegetation on earth (Batra, 1995).

Michener (2007a) reported that the vast majority of bees in the world were solitary and in their study, solitary bees in the family Halictidae were the most species-rich, but individuals reported were rare (74 individuals, 20 species). The labor of nest construction and provisioning, foraging and egg-laying was all done by single, fertile female bees. Plant diversity in floral shape, size, colour and fragrance has evolved to attract pollen bees. Bees could live everywhere, with the greatest abundance and diversity in semi-arid and warm temperate climates. Solitary bees were most diverse and abundant in deserts, prairies and other undisturbed natural habitats (Michener, 2007a). Many solitary bees did not resemble honeybees, instead, they looked alike wasps, bumble bees, or flies. Solitary pollen bees were coming under the superfamily Apoidea and could be further classified into seven families *viz*., Apidae, Halictidae, Megachilidae, Colletidae, Andrenidae, Melittidae and Stenotritidae (Michener, 2007a). The membrane bees which were coming under the family Colletidae were the most

numerous and diverse in the Southern hemisphere; the Andrenidae or digger bees were present mainly in the Northern hemisphere; the Halictidae or sweat bees were distributed worldwide; the Megachilidae or leafcutter and mason bees also appeared worldwide and the Anthophoridae, carpenter and miner bees predominantly were observed in the tropical. There were some other subfamilies consisting of solitary bees such as Oxaeinae, Fideliinae and Xylocopinae.

Although honey bees were considered the most adaptable and commonly applied managed pollinator to enhance the production of different crops, this species was not the only insect that could pollinate plants of commercial value (Allsopp *et al*., 2008). Wild insect pollinators other than honey bees have been recently recognized for their role in improving and stabilizing crop pollination services because fruit sets significantly increased with the visitation rate and species richness of wild pollinators, mainly native solitary bees (Mallinger and Gratton, 2015). In contrast, there was no relationship between honey bee abundance and seed set. Similarly, pollination limitation decreased significantly (lower values of pollination limitation indicate natural bee pollination closer to the maximal applied by hand) with increasing wild bee species richness and marginally decreased with wild bee species abundance but had no relationship with honey bee abundance (Blitzer *et al*., 2016).

Blitzer *et al*. (2016) found that the wild bee community was numerically dominated by solitary, ground-nesting bees in the genus *Andrena* (Andrenidae), which accounted for 62 per cent (594 individuals, 18 species) of all wild bees collected.

2.1.4.1 Solitary bees recorded in India

In India, Bingham (1897) and Batra (1977) conducted important taxonomic works on bees. In Kerala, Jobiraj (2002) studied the systematics of the bee family Apidae. Nayana (2008) recorded 46 species of non-*Apis* bees coming under 13 genera while Dhanyavathi (2009) recorded 55 species of non-*Apis* bees under 16 genera. Pannure *et al*. (2017) published a distributional checklist of Nomiinae of South India and recorded 48 species under 13 genera. Sheeja and Jobiraj (2017) conducted studies on bee fauna of the Vanaparvam Biodiversity Park, Kozhikode, Kerala and recorded 18 species under 9 genera. According to Pannure *et al*. (2017), a total of 796 species of

bees under 71 genera were recorded in India. Bijoy *et al*. (2019) recorded 19 species of bees belonging to 7 genera from the rice ecosystems of Palakkad.

2.2 Importance of bee pollination in cucurbit ecosystems

Alex (1957) reported that honey bees were the most important pollinator of cucurbit crops and a few solitary bees were also found to be effective pollinators. Bhambure (1958) observed *Apis florea* and *Melipona* sp. pollinating cucumber fields near Mumbai and also reported that monoecious flowers of ridge gourd, *Luffa acutangula* L., were visited vigorously by *Apis cerana*, *Apis florea* and *Melipona* sp. from 09.00 to 11.30 h. Batra (1967) reported that *Apis flora* Fab. visited more flowers in *Cucurbita maxima* L. as against *Apis dorsata* Fab. The other pollinators such as *Nomia oxybeloides* Smith, *Lasioglossum cattulum* Vachal, *L. massuricum* Bluth, *Nomioides minutissima* Rossi, *N. variegate* Oliver and *N. divisa* Cameron was in negligible numbers.

Hurd *et al*. (1971) reported that honeybees only managed to scrape pollen from the anthers with great difficulty and their pollen loads were very small as compared to those of solitary bees coming under the genera *Peponapis* and *Xenoglossa* which visited flowers earlier in the day when the pollen was first available. Grewal and Sidhu (1978) observed that solitary bees belonging to families, Anthophoridae, Xylocopidae, Megachilidae and Halictidae were important pollinators of *Cucurbita pepo*. It was reported that honey bees increased yields in cucumber and other vine crops (McGregor, 1976). Other insects such as thrips and beetles (Rosa, 1925), ants (Tontz, 1944) and solitary bees (Jaycox *et al*., 1975) have been identified as possible pollinators of cucurbits. The honey bees and the halictid solitary bees were the most abundant bee visitors (70 % and 23 % respectively) of muskmelon in Punjab (Grewal and Sidhu, 1978). Girish (1981) observed that *Apis cerana*, *A. dorsata* and *A. florea* were the major pollinators of summer squash which contributed 87, 10 and 3 per cent altogether in pollination respectively near Bengaluru. The fruit number and fruit weight were higher in honey bee pollinated crops compared to open pollination (Rao and Suryanarayana, 1988). Shrivastava (1990) studied 23 species of insect pollinators visiting cucurbitaceous crops in Rewa, India and concluded that *Xylocopa fenestrata* Fab. was the best pollinator for white flower gourd/bottle gourd, *Luffa siceraria* Standl.

Rajasekhar (2001) observed the effect of bee pollination on watermelon. He reported that significantly higher fruits per 30 m^2 were recorded with two colonies per plot (22.37) followed by one colony per plot (20.75) and the lowest with no colony (18.37). Similar results were also obtained concerning mean fruit weight, fruit diameter, total soluble sugar per cent and yield. Eswarappa *et al*. (2001) reported 26 insect pollinators visiting chow-chow crop, under which 14 insects belonged to Hymenoptera and 4 each to Diptera, Lepidoptera and Coleoptera. *Apis florea*, *A. dorsata* and *A. cerana* comprised more than 80 per cent of the total insect visitors of chow-chow during the study. Kumar (2002) opined that a minimum of eight bee visits per flower was necessary for pumpkin to get fruit set, fruit weight, fruit volume and several sound seeds per fruit. There was no fruit set in watermelon plots excluded from insect pollinators. Larsson (2005) suggested that the pollinator effectiveness of a specialist solitary pollen bee could be superior while opportunistic flower visitors selected floral characters towards generalization through their contribution to overall pollen flow.

Kuberappa *et al.* (2006) demonstrated that fruit weight (5066.25 g), fruit volume (4985.00 ml) and pulp ratio (10.28 %) were maximum in open pollination of pumpkin compared to other modes of pollination. Hand pollination either at 09.00h or 10.00h did not make any significant differences in the per cent fruit set, fruit weight, pulp ratio and seed germination. The cucurbit crops were established as one of the largest groups among the vegetable crops (Nath, 2007) with their wide adaptation from arid to humid tropic environments. The Asian and Pacific regions were reported to produce many edible cucurbits and it was considered to be the center of origin for some of them. Yang *et al.* (2007) reported that muskmelon fruits pollinated by honey bees were smaller compared to those by bumble bees and that the fruits pollinated by bumble bees were with higher soluble solids. Santos *et al*. (2009) found that bitter gourd, watermelon, cucumber, and luffa crops were visited by stingless bees, which were only a small proportion of the complex of visitors.

Bodlah and Waqar (2013) recorded several bee species pollinating the cucurbitaceous flowers at Ludhiana. Of the various bees, *Apis dorsata* was the most abundant followed by *A. florea*, *Ceratina binghami* Cockerel, *Xylocopa pubescens* Spinola, *Nomioides* sp. and halictid bees. The number of sound seeds per fruit

(258.25) and seed weight (52.65g) were maximum in hand pollination at 09.00 h compared to other modes of pollination. Garantonakis *et al.* (2016) reported that several species of solitary bees were found visiting watermelon flowers but honey bees were found as the principal pollinator.

2.2.1 Insect pollinators of bitter gourd and oriental pickling melon

McGregor and Todd (1952) observed that melon flowers hardly set fruit until the bees were introduced into the caged melon plot. However, there was a rapid fruit set resulting in 184 marketable melons when the bees were introduced into the cage. In the open plots of melons, only 145 fruits were produced against the caged plots. Thus it was suggested that a sufficient bee population should be present in the crop field to get maximum yield. Seyman *et al*. (1969) stated that bees were extremely good pollinators of cucumber crops and the major portion of bee pollination activity occurred during the mid-day period. Rao and Suryanarayana (1988) reported that *A. cerana* was the principal pollinator of the cucumber crop and was found to be an efficient pollinator than *A. florea* and *T. iridipennis*.

Cervancia and Bergonia (1990) observed that common flower visitors of the cucumber were *A. dorsata*, *Xylocopa chlorinae* Smith, *X. philippiinensis* Smith, *Megachile atrata* Smith and they were most abundant during 10.00 to 11.00 h. Fruit set in insect-pollinated (78 %) and hand-pollinated (80 %) flowers did not significantly vary in bitter gourd (Free, 1993). Likewise, there was no significant difference in fruit weight, length, diameter, and some seeds between both methods. A study on cucumber plants by Prakash *et al*. (2001) revealed the presence of 27 insect visitors in which 16 pollinators belonged to Hymenoptera and 4 each to Diptera, Lepidoptera and Coleoptera. Kumar (2002) observed that hymenopterans were the major flower visitors of bitter gourd in Hisar, Haryana, in which *Halictus* sp. was the most frequent flower visitor (43.88 %) followed by *Megachile* sp. (32.11 %) and *Apis dorsata* (24.01 %). Sajjanar *et al*. (2004) showed that among the 24 flower visitors of cucumber reported during his experiment, *Apis dorsata* was the most frequent insect pollinator of cucumber plants followed by *A. cerana, Tetragonula iridipennis* and *A. florea*.

Nidagundi and Sattagi (2005) observed that *Apis florea* was the most predominant pollinator in bitter gourd, constituting 43 per cent of the total pollinators.

Flowers that were not visited by pollinators set no fruits (Deyto and Cervancia, 2009). Rubina (2010) reported that, among the 28 species of insect pollinators that visited cucumber plants, 20 belonged to Hymenoptera, in which honey bee species contributed 84 per cent of total pollinators.

Subhakar *et al*. (2011) reported that *Tetragonula iridipennis* (86.31 %), *Halictus gutturosus* (8.68 %) and *A. florea* (3.84 %) were the most frequent and abundant flower visitors of bitter gourd in Tirupathi. Balina *et al.* (2012) found that *A. dorsata* was the most efficient pollinator followed by *Halictus* sp. and *Megachile* sp. among the nine flower visitors of bitter gourd in Haryana.

According to Oronje *et al.* (2012) fruit set and yield were pollen limited, as all bagged flowers were aborted. Fruit set under natural pollination was very low which revealed the degree of pollen limitation in *M. charantia*. The low fruit set was consistent with the observation of high discrimination against pistillate flowers amongst potential pollinators.

2.3. Determination of pollinator performance

McGregor and Todd (1952) opined that insect pollinators had their specific ecological threshold level, below which they stopped their activity. Whittaker and Bohn (1952) observed that honey bees showed variation in visiting flowering plants according to the microclimate around them, which resulted in receiving more flower visits than necessary to some plants. Sanduleac (1959) reported that honey bees could work most intensively from 06.00 h to 12.00 h with a maximum activity from 08.00 to 9.00 h on cucurbit flowers in Rumania. He found that honey bees worked more vigorously on staminate flowers than pistillate flowers.

Nemirovich-Danchenko (1964) from Siberia and McGregor *et al*. (1965) from Russia reported that bees collected nectar from cucumber flowers between 10.00 to 12.00 hours. Conner (1969) claimed to ensure cucumber pollination with eight bee visits per flower. Seyman *et al*. (1969) reported that the general foraging activity of honey bees was noticed throughout the day, but peak activity was observed between 08.00 to 11.00 h in winter, 06.00 to 11.00 h and 16.00 to 18.00 h in summer and 08.00 to 12.00 h in monsoon irrespective of the crops in the transitional area. Affirming that, Stephen (1970) observed *Halictus* sp., which made an average of eight visits per

blossom that was required to yield uniform-sized cucumber. Stebbins (1970) formulated the most effective pollinator principle, where he suggested that plants should always be evolved with specializations to their most effective pollinator. Kauffeld and Williams (1972) showed that honey bees collected nectar throughout the day from cucumber flowers with peak foraging activity from 11.00 h to 14.30 h.

Gary *et al*. (1975) found that 13^0C appeared to be the minimum threshold temperature for the initiation of field activity by the honey bees. Some specialist bee species were known to be more efficient in flower handling on their preferred host plant than generalist bee species (Strickler, 1979). The ecological threshold required for normal activity differed greatly depending on the level of adaptability of a species in a given environment (Kapil and Jain, 1980). Schemske (1980) pointed out that pollinator effectiveness was promoted by floral traits that in turn exerted a positive selective pressure on pollinator fitness, through which the coevolution occured. Motten *et al.* (1981) observed that the specialist bee *Andrena erigeniae* had similar pollinator effectiveness to the generalist fly *Bombylius major* (Bombyliidae) in *Claytonia virginica* (Portulacaceae).

Lerer *et al*. (1982) pointed out that though ambient temperature played an important role in the initiation of flight, solar irradiance was primarily responsible for controlling flight activity and cessation of activity, even before the temperature dropped to the level required for initiation of bee activity. The pollen collecting insects were considered more valuable pollinators than nectar collectors (Mohr and Jay, 1988). Sihag (1988) stated that the foraging behavior of the pollinators depended upon the shape and size of flowers they visited and a visitor was said to be a pollinator if it intentionally or unintentionally transferred pollen from anthers to the stigma in a foraging attempt. On the other hand, if it did not transfer pollen, was considered a non-pollinator. Oh and Woo (1990) suggested that insect activity increased sharply after sunshine and decreased gradually through the day and ceased before sunset.

According to Thomson and Goodell (2001), bee species could vary in the number of flowers they visited per time and in their efficacy at depositing or removing compatible pollen on the stigma per visit. Eswarappa *et al*. (2001) observed that the activity of different species of honey bees either in an open or caged plot of chow-chow was found to be maximum at 10.00 to 11.00 h and the lowest at 06.00 h. He also

found that the peak foraging activity was at 10.00 h and the time spent by honey bees for collection of pollen was maximum between 08.00 h to 09.00 h. Monzon *et al*. (2004) and Larsson and Franzen (2007) discovered a linear relationship between critical pollen resources and the size of the bee population. They also observed that the foraging behavior of *Andrena hattorfiana* required long periods of suitable warm and sunny weather to succeed in collecting the amount of pollen needed to provision one nest. Pollinator performance of solitary bees and flies have been compared by Larsson (2005) and revealed higher effectiveness of solitary bees in gynodioecious herb, *Knautia arvensis*. They noted that females of specialist solitary bee *Andrena hattorfiana* accounted for 14.2 per cent of total visits and 5.8 per cent of the total pollination, the rest being performed by generalist plant visitors and males of *A. hattorfiana.* Nidagundi and Sattagi (2005) studied the relevance of bee pollination in bitter gourd yield through cage experiment, which revealed that maximum fruit length (26.10 cm) in bitter gourd was obtained with honey bees to fruit length in open pollinated (13.93 cm) and caged plots (13.60 cm). The highest pulp ratio (0.132) and the highest fruit weight (129.21 g) were also observed in bee pollinated plants while, the pulp ratio was 0.09, and 0.07 and fruit weight was 72.09 g and 62.44 g in the open pollinated and caged plot without bees, respectively.

Nicodemo *et al.* (2009) reported that *Trigona* sp. spent a mean time of 60.50s per flower in Brazil. Maximum time spent by *Trigona* sp. on a flower could be related to lower visitation rates in terms of energy kinetics. Higher visitation rates coupled with the moderate foraging speed of *Halictus* sp. and *Apis* sp. (09.00 and 11.00 sec/flower, respectively) contributed to effective pollination qualities. Deyto and Cervanica (2009) reported that flowers that were not visited by pollinators set no fruits. The activity of honey bees was the highest between 11.00 to 12.00 hours when the temperature averaged from 21^0C to 25^0C (Rubina, 2010). They also observed that the quantity of nectar and sugar concentration gradually increased with the advancement of time and reached the maximum at 15.00 h in both male and female flowers of cucumber and thereafter decreased towards the end of the day. The quantity of nectar was more in female flowers (2.34 µl) than in male flowers (1.80 µl) but the sugar concentration was more in male flowers (37.31 %) than in female flowers (31.40 %). Similarly, peak nectar foraging activity of honey bees was found at 12.00 h of the day and the time spent by different honey bee species in the collection of nectar from

pistillate and staminate flowers was found to be maximum at 13.00h. The maximum time spent was by *A. florea* (306.30 sec/flower), followed by *T. iridipennis, A. dorsata* and *A. cerana* with a mean time spent of 295.32, 36.07 and 35.65 sec/flower, respectively.

Oronje *et al*. (2012) showed that fruit set under natural pollination was very high in bitter gourd, thus establishing that fruit set and yield were pollen limited as all bagged flowers were aborted. Srikanth (2012) reported that maximum fruit weight (1.87 kg), fruit volume (2340 ml), fruit length (43.93 cm) number of sound seeds per fruit (423) and seed weight (16.58g) in bottle gourd were recorded in open pollinated plot with attractants compared to crop caged without bees (69.43g) which indicated that pollinators were must for higher fruit set in bottle gourd.

Balina *et al*. (2012) observed that the foraging rate of *Halictus* sp. varied from 4.05 to 8.65 flowers during different hours of the day in bitter gourd. Jauker *et al*. (2012) showed that post-flowering parasitoid pressure and a sudden shortage of nectar decreased the life span of solitary pollen bees. Their physiological restrictions limit their activity to certain abiotic conditions (Rader *et al*., 2013). Bischoff *et al*. (2013) found that pollinator performance was determined primarily by visitation frequency rather than assessing their pollen deposition effectiveness. Brosi and Briggs (2013) showed that the loss of a single species could reduce the pollination functioning of the ecosystem, even under the presence of other potentially efficient pollinators. Lakshmi (2013) reported that the maximum foraging activity of *A. cerana, A. florea* and *T. iridipennis* in ridge gourd was between 09.00 and 11.00 h of the day whereas, the other pollinators were observed between 12.00 and 16.00 h. The time spent for nectar and pollen foraging by *A. cerana, A. florea* and *T. iridipennis* was maximum at 09.00 and 11.00 h of the day. Bodlah and Waqar (2013) also recorded a much higher foraging rate in the early morning *i.e.,* 06.00 to 07.00h in ridge gourd, bitter gourd and eggplant. Solitary bee species richness showed a positive correlation with the availability of species-rich grassland and they could improve the fruit yield in orchards (Woodcock *et al*., 2013).

The studies carried out by Subhakar and Sreedevi (2015) showed that though the number was very less in the case of *H. gutturosus* and *A. florea*, the foraging rate was higher increasing its pollination efficiency and despite *T. iridipennis* being high in

bitter gourd ecosystem, its pollination efficiency was lesser. They also studied the foraging speed of major bee species on bitter gourd flowers and found that the maximum time spent by *T. iridipennis* was 89.20 sec. with a mean time of 40.62 sec. per flower followed by *H. gutturosus* which spent maximum and minimum time of 31.20 and 4.20 sec., respectively with a mean foraging time of 10.80 s per flower. Among all three major pollinators, *A. florea* spent less time on flowers. The maximum time spent by *A. florea* was 14.26 sec. with a mean foraging time of 9.28 sec. per flower. This was recorded during the peak foraging hour of all three bee species.

Subhakar and Sreedevi (2015) revealed that *T. iridipennis* started their foraging activity at 06.00 h of the day and their mean number increased up to 10.00 h and thereafter declined with maximum foraging activity at 09.00 h (24.41 bees / m^2 / 5 min) followed by 10.00 h (21.40 bees / m^2 / 5 min.). Moderate foraging activity with 15.84 bees/m^2/5 min and 14.01 bees / m^2 / 5 min were observed at 08.00 h and 11.00 h, respectively. Less activity was observed in the early morning hours and midday hours ranging from 1.60 to 5.17 bees / m^2 / 5 min. The foraging activity of *T. iridipennis* decreased with its minimum at 13.00 h (1.00 bees / m^2 / 5 min.). Davidar and Carr (2015) pointed out that levels of pollinator dependency ranged from zero per cent in tomatoes to 76 per cent in bitter gourd thus, affirming the importance of pollinators in fruit sets of bitter gourd plants. The number of functional groups appearing at a site was the strongest predictor of increased pollination services and greater pollinator functional diversity could lead to an improved seed set (Blitzer *et al*., 2016). Yogapriya *et al*. (2019) reported that *Trigona* sp. spent maximum time on a bitter gourd flower followed by *Halictus* sp. and *A. florea*.

2.4. Nesting preferences of solitary pollen bees

The process of securing competent pollinators to ‘service' agricultural areas is proving challenging to design and there is a revived interest in assisting nature in providing pollination services by promoting wild pollinator habits.

Sakagami and Michener (1962) reported that the nesting architecture of ground-nesting bees varied according to species. Typically, nests were composed of a main vertical gallery with lateral tunnels leading to ovoid brood cells having one offspring per cell. Krombein (1967) reported that it was difficult to locate the nest of native

solitary bees since most of the time they remain unperceived by our eyes. De-Lello (1971) reported that the Dufour's gland of the colletid bees produced cell lining secretions of their nest, which was associated with the sting apparatus. Kamm (1974) observed that most ground nesters constructed multiple cells per nest and cell size were positively correlated with bee body size.

Stephen (1981) and Rank and Goerzen (1982) observed the nests of alfalfa leafcutting bee with linear series of cells delimited by cut-leaf partitions. Tepedino and Torchio (1982) and Richards (1984) found that the cavity-nesting mason bee *Osmia lignaria* averaged 1.8 nest cells completed per day in a greenhouse stocked with excess bloom. Packer *et al*. (1989) found that lateral tunnels of a typical ground nest varied in length and angle at which they connected to the main shaft. Wcislo *et al* (1993) observed that some ground-nesting halictid bees had no lateral tunnels in their nest, instead their brood cells were directly connected to the main shaft. Martins and Antonini (1994) reported that ground-nesting female bees initiated nest excavation using their mandibles or their forelegs and pushed out the soil using their hind legs and abdominal movements. Arriaga and Hernandez (1998) showed that the members of families Apidae and Megachilidae occupied the wooden trap nests while the Colletidae seldom occupied the nests. Gosek (1999) reported that several wood-nesting bees of *Megachile* and *Osmia* spent 5 - 17 days constructing and furnishing the nest and cells. Michener (2000) reported that nests were essential for the reproductive success of bees because they sheltered their brood and provided essential conditions for the development of their immature by being inside the nest. Construction of brood cells was important and the generalized steps when setting up cell walls comprised of; (1) lining the surface with a smooth earthen layer, (2) tamping the cell surface smooth with the pygidial plate and (3) applying a secreted film of wax-like or cellophane-like material to the earthen surface (Michener, 2000).

Kemp *et al*. (2004) found that adult females of mason bee, *Osmia rotundata* (L.) were active for approximately 20 days in the spring, during which they built one or more nests in the abandoned beetle burrows in dead timber or vacant nests of other bees (mainly *Anthophora* sp.) in clay embankments. In addition to foraging for pollen and nectar to provision their nests, females also collected mud to build cell partitions and to seal completed nests. Zillikens and Steiner (2004) observed that the leaf cutting

bee *Megachile pseudanthidioides* built their nests in borings of hardwood blocks, in small wooden boxes and in bamboo canes. The brood cells consisted of an outer layer of overlapping small leaves or pieces of leaves cut into oval pieces of flower petals that served as the innermost lining of the cells. Most bees build their nest in soil, wood, hollow stems, pithy stems, or pre-existing cavities and tunnels abandoned by other wood-boring insects (Potts *et al*., 2005). Morato and Martins (2006) mentioned that intrinsic and extrinsic factors affect the cavity occupation and preference of the bees. Kind of cavity, animal size to hollow entrance size, cavity position on the tree, orientation, thermoregulatory capacity and social organization of the animal species certainly influence cavity occupation and use. Many solitary species depend on pre-existing above-ground cavities or specific soil microhabitats often associated with semi-natural habitats (Cane *et al*., 2007). Native bees were reported to exhibit diverse requirements for the habitat and type of substrate they use for their nest construction (Danforth, 2007).

Winfree (2010) mentioned that although the ground-nesting strategy was observed in solitary and social bees, ground-nesting bees were proportionally far less studied than cavity nesters. Zurbuchen *et al.* (2010) showed that energy and time invested in nest site selection and nest construction required potential fitness costs and this meant that nest-site selection might play a key role in female bee fitness. According to Cane and Neff (2011), the nest of most ground-nesting bees has not yet been described, but based on a worldwide review of 449 species, cell depth could range from 1 to 530 cm with an average cell depth of 17 cm beneath the soil surface. Roulston and Goodell (2011) reported that female ground-nesting bees did not care for their brood after provisioning and sealing the cells. Thus they constructed their nests by providing high-quality food and high-quality location to maximize the success of their brood. McKinney and Park (2012) observed that the Japanese horn faced bee, *Osmia cornifrons* Radoszkowski gathers pollen by consuming more time, requiring 221.6±28.69 minutes per cell and cell provisioning was the most time consuming intra-nest activity, requiring 28.9±3.97 minutes. According to Yoon *et al*. (2015), *Osmia* bees are the cavity nesting solitary bee species that play an important role as pollinators.

Ali *et al*. (2016) reported that *Ceratina smaragdula* preferred pruned stalks Ravenna grass for nesting. Danforth *et al.* (2019) mentioned that, unlike cavity nesters, ground nesters had specialized pygidial (on the sixth segment of their abdomen) and basitibial plates, which allowed the bees to dig, pack soil and move easily within the nest. Leonard and Harmon-Threatt (2019) opined that the life cycle of cavity-nesting bees could be observed using artificial nesting structures whereas, ground-nesters seemed to be difficult to locate and only very few methods were reported to study the within-nest behavior of these bees. Ground nesters chose nesting sites that were protected from adverse weather conditions, excessive humidity, and incidental risks such as predators and parasitoids.

Kaliaperumal (2019) reported that the wild solitary bee *Ceratina hieroglyphica* Smith, preferred the pithy region of dried cashew twigs as their nesting sites, compared to the fresh twigs or already made burrow holes by other wood boring insects. Udayakumar and Shivalingaswamy (2019) studied the nesting preference of the small carpenter bee *Ceratina binghami* Cockerell, which revealed that they constructed a nest in dead woods and pruned pithy stems by making linear burrows. Latha *et al.* (2020) observed that six different plants with pithy stems were preferred as nesting hosts for *C. binghami viz*., crotons, yellow bell, oleander, peacock flower, copper pod and rose.

2.5. Nesting architecture and life cycle of solitary bees

Richards (1984) studied the life cycle of an alfalfa leaf-cutting bee, where they observed that the bees deposited an egg on top of the provisioned mass of pollen and nectar. Completed nests were sealed with a cut-leaf plug. By the late summer, the fifth instar larvae completed consumption of the pollen-nectar provision, defecated and spun a cocoon with silk-like strands. In the pre-pupal stage, most bees underwent a diapause period that lasted through the winter months and under natural conditions completed their development through the adult stage and emerging temperatures increased during the following spring and early summer.

Small carpenter bee, *Ceratina binghami* constructed their nests in dried tiny twigs and pruned pithy stems by making linear burrows in peacock flower trees, *Caesalpinia pulcherrima* (L.) (Fabaceae) (Udayakumar and Shivalingaswamy, 2019)

and *C. hieroglyphica* found constructing their nests in the pithy region of dried twigs of the cashew tree, *Anacardium occidentale* (L.) (Anacardiaceae) (Kaliaperumal, 2019). The females of *Ceratina* chewed the central pith of selected twig, and flies out to forage pollen and nectar. They mould the collected pollen into pollen masses to oviposit on them and closed the cell by septum (McIntosh, 1996). Mothers found to inspect the brood cells constructed by them and mostly found in the gallery between the entrance and the first brood cell often in a defensive position blocking the nest entrance to protect the broods from natural enemies (Rehan and Richards, 2010).

2.6. Natural enemies of solitary bees

Ricketts *et al*. (2008) found a decline in pollinator richness and native bee visitation rate to flowering plants with increasing distance from their natural habitat. Kremen *et al.* (2007) mentioned that the temporal and spatial availability of food, nesting sites, overwintering and mating sites of pollinators were strongly influenced by the landscape structure. Thus, changes in land use and landscape structures could affect the plant-pollinator interactions and individual pollinator populations in the community.

Torretta *et al*. (2012) found that approximately 30 per cent of all offspring of *Megachile gomphrenoides* failed to complete development to the adult stage and an additional 10 per cent were killed by their natural enemies. These included parasitic wasps (Eulophidae: *Melittobia* and *Horismenus*), a cleptoparasitic bee (Megachilidae: *Coelioxys*) and a blister beetle (Meloidae: *Tetraonyx*). Shebl *et al*. (2018) reported several natural enemies of the solitary bee, *Osmia latreillei,* which were collected in front of the artificial nesting sites included, cleptoparasitic bees and wasps, beetles, predatory ants, larvae of some unidentified parasites and predators and several pathogens. Kaliaperumal (2019) reported *Neochalcis* sp and *Beauvaria bassiana* as the natural enemies in the nest of solitary bee, *C. hieroglyphica*.

2.7. Palynology

The word “palynology” was coined by Hyde and Williams (1945) as a substitute for “the science of pollen grains and spores”. Bohart (1955) and Taniguchi (1956) dissected a broad variety of female solitary pollen bees, and found that their crops and midguts contained a visibly abundant quantity of pollen. It was reported that

dietary pollen extended the longevity and fecundity in pollen-feeding insects, which had been shown for adult female seed beetles (Leroi, 1981) and thrips (Kirk, 1985). Like most butterflies and moths, only the adults of these various insects fed from flowers. Sarviva (1985) reported that the daily flight activity of insect pollinators varies with time and meteorological variables, especially wind, rainfall, humidity and temperature. The population density of insect pollinators on blossoms depends upon nectar sweetness and weather conditions (Seeley and Levien, 1987). According to Nachtigall *et al.* (1989), bees are the quintessential floral foragers, both for self-maintenance and for acquiring food for their offspring and the nectar sugars power bees' flight.

Pollen feeding by diverse adult insects is far less commonly documented than nectar drinking (Roulston *et al*., 2000). Adult female bees collect pollen from flowers, usually transporting it externally in hairy scopal brushes for deposition at the nest (Thorp, 2000). Palynology, the scientific study of pollen and identification of its origin, plays an important role in studying mechanisms of plant-pollinator interactions (Wilcock and Neiland, 2002). Adult female heliconiid butterflies extract essential amino acids from collected pollen, which ends up in their eggs (O'Brien *et al*., 2003). Mass-flowering crops have been suggested to be important for mitigating pollinator declines in modern agroecosystems due to their ample provision of foraging resources (Westphal *et al*., 2003). Cane and Sipes (2006) reported that, unlike most bumblebees, many solitary bees are oligolectic, since they gather pollen from plant species that belong to a single family. Females of most bee species also blended the collected nectar into larval diets (Michener, 2007a). Several case studies pointed out that pollen feeding by some insects improved their longevity and reproductive output (Wackers *et al*., 2007). Solitary bees generally lay only 1-2 eggs daily (Neff, 2008), even with unlimited bloom and long foraging days in an ideal greenhouse environment. Cane (2008) showed that females of a gregarious solitary pollen bee *Nomia melanderi* Ckll. eat several large meals of pollen throughout their life cycle, to maintain their reproductive ability. Pollen grains often display a species-specific morphology, with diverse structure and sculpture (Fall, 2010). Pollen constitutes a substantial fraction of the larval bee's diet too (33 % for *Megachile rotundata*) (Cane *et al*., 2011). In contrast, pollen feeding by adult bees is rarely reported and poorly understood (Rader *et al*., 2013).

Woodcock *et al*. (2013) found that solitary bees showed the greatest probability of achieving stigmal contact with their bodies which was probably influenced by a combination of the greater average time spent on individual flowers as well as their tendency to collect pollen as opposed to just nectar. Cane (2016) mentioned that every female solitary bee is fertile and bears the nutritional cost of maturing eggs. Unlike honey bees, very few species of solitary bee are known to augment larval provisions with glandular secretions. Cane (2016) also observed that the yolk lipoproteins (vitellogenins) invested in their eggs must come from a dietary nitrogen source, which for bees is pollen. Conceivably, their vitellogenins could be synthesized from pollen proteins held over from the larval stage or else gained during adult pollen feeding.

Shebl *et al*. (2018) observed that female solitary bees started constructing their nests after mating. Females started to collect several loads of pollen grains to provision the recently completed cell. Once a brood cell was supplied with appropriate provisions, females were observed making a final flower-visiting trip during which she collected only nectar. Upon returning to the nest, the female regurgitated the nectar onto the pollen. The female collected about 2-4 mud loads carried between the mandibles to close the nest, leaving a space between the upper cell and the nest entrance. The first instar remained partially within the split chorion and continued feeding on the egg's fluids until molting to the second instar. Once the larva developed into the second instar, it emerged completely from the egg and began feeding on the pollen-nectar provisions. For these and other reasons, the role that pollen feeding plays in reproduction by adult solitary bees cannot be extrapolated from the shifting, caste-specific pollen feeding needs of sterile worker honey bees.

2.8. Effect of plant protection measures on pollination

In highly disturbed habitats, such as agricultural landscapes where the use of herbicides or pesticides is common, plant and pollinator diversity was found much lower than in other less disturbed habitats. (Rathcke and Jules, 1993; Kearns and Inouye, 1997; Kevan *et al*., 1997; Kearns *et al*., 1998).

Historically, there had been a trade-off between achieving food sustainability and conserving biological diversity, a concern that was most prominent in the

developing world where, the ever-increasing population stressed finite biological resources (Abalu and Hassan, 1998).

Morse and Calderon (2000) reported that 80 per cent of total pollination in plants is contributed by insects. Ecosystem stability and diversity in plant assemblages are essential features of the development and maintenance of the pollinator's guild. The number of solitary bee species has declined substantially throughout Europe as a result of the loss of meadows, ditching, pesticides, paving of country roads and large-scale farming units (Carvell *et al*., 2006).

Klein *et al.* (2007) opined that habitat loss, increased use of conventional agrochemicals and intensified agricultural practices threatened vital ecosystem services provided by wild bees. Laboratory and field toxicity tests on managed bees showed that even sublethal effects of pesticides on individual bees could have ramifications for bee populations (Desneux *et al*., 2007).

Wu *et al*. (2011) reported that bee larvae exposed to pesticides during their developmental stages would take more time to complete their life cycle and showed reduced longevity. Marini *et al*. (2012) found that native bee abundance and diversity were low in orchard-dominated landscapes because of the increased distance to the orchard interior from peripheral bee habitats, seasonal pesticide and fungicide applications and lack of floral resources when apples are not in bloom. Though studies on the effect of pesticide exposure on wild bees were hardly done, field comparisons of organic and conventional farms have been the first to reveal measurable effects of increasing pesticide use on wild bee communities (Kennedy *et al*., 2013).

Mitchell *et al*. (2017) conducted a study for analyzing the presence of neconicotinoid pesticides in honeybee hives, in which they found that all the honey and pollen samples were containing 75 per cent of neonicotinoides. Siviter *et al*. (2018) reported the impact of pesticides on bee learning and memory across a range of dosage regimes and pesticide treatments. They concluded that though the magnitude of the impact of neonicotinoides over other pesticides in bee learning and memory was unable to be distinguished, chronic pesticide exposure had a greater impact on bee memory than acute exposure.

Materials and Methods

3. MATERIALS AND METHODS

The present study entitled "Pollination ecology of solitary pollen bees" was carried out at the Department of Entomology, College of Agriculture, Kerala Agricultural University, Vellanikkara from 2018 to 2021. The experimental area experienced a typical warm humid tropical climate and located at 10.54°N and 76.27°E at an average elevation of 23m from MSL. The site had received an average rainfall of 3198 mm annually. The temperature of the region ranged from 20°C to 39°C and the average relative humidity of the region varied from 47 per cent to 89 per cent where, maximum per cent of relative humidity was observed in July-October (70-90 %) and minimum per cent of relative humidity was observed during December-March (50-60 %). The details of materials used and the methodology adopted for the investigation of research programme are furnished here under.

3.1. Documentation of pollinator diversity in selected cucurbitaceous crops

3.1.1. Roving survey for the collection and documentation of pollinators

Roving surveys were conducted in Central districts of Kerala at monthly intervals from March 2018 to December 2019. All the surveys were conducted when the selected cucurbitaceous crops such as bitter gourd (*Momordica charantia* L.) and oriental pickling melon (*Cucumis melo* var. *conomon* Mak.) were at ≥50 per cent blooming period. Sweep nets were used to collect the flower visitors. Insects collected in sweep nets were transferred to polythene bags, labelled and brought to the laboratory. The major flora and its associated bee fauna in and around the survey area were recorded.

3.1.2. Sample collection

Pollinators were collected from all three central districts of Kerala *viz*., Palakkad, Thrissur and Malappuram. Surveys were conducted between morning 7.00 AM to evening 3.00 PM. The pollinators were collected from bitter gourd and oriental pickling melon ecosystems using the sweep net sampling method. Sweep net samples were immediately transferred into polythene bags of size 24×36×24 cm with proper labels and collected pollinators were killed using ethyl acetate. The number of insect pollinators collected, the number of individuals in each species, and the name of host

plants from which they were collected were recorded for working out the diversity indices of all pollinator species.

3.1.2.1. Preservation of specimens

All specimens were preserved either dry or wet, neatly labelled and stored in insect boxes.

3.1.2.1.1. Preservation of specimen in dry condition

Specimens from each sample were taken out and kept in a desiccator for one hour to reduce the stiffness of the samples. Specimens were properly spread and pinned using entomological pins in such a way that all the diagnostic characters were easily visible. The pinned insect specimens were kept in a hot air oven at 45°C for drying. Singleton species obtained during surveys were preserved in dry conditions only.

3.1.2.1.2. Preservation of specimens in wet condition

The insect samples were wet preserved in 70 per cent ethyl alcohol solution in Eppendorf vials, properly labelled and stored in a deep freezer at 4°C for further identification and analysis.

3.1.3 Calculation of diversity indices and abundance

Month-wise data on all pollinators collected from Central districts of Kerala were compiled for carrying out the statistical analysis. All the statistical analyses on diversity indices were done using the PAST (Paleontological Statistics Software Package for Education and Data Analysis) version 4.04.

3.1.3.1. Measuring species richness

Species richness is considered the direct estimation number of species in the study site (Magurran, 2004). Margalef's diversity index (Clifford and Stephenson, 1975) D_{Mg} and Menhinick's index (Whittaker, 1977) D_{Mn} were used to calculate the species richness of pollinators using the formulas,

$$D_{Mg} = \frac{(S-1)}{\ln N}$$

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

Where, S = Richness (*i.e.* number of species collected)

N = Number of individuals recorded in the sample.

3.1.3.2. Diversity and evenness indices

Species diversity was calculated using Shannon-Weiner diversity index (*H′*) or Simpson diversity index (*D*).

Shannon Index was calculated using the formula,

$$H' = -\sum P_i l_n P_i$$

Where P_i = Proportion of individuals in the i^{th} species

$$D = \sum P_i^2$$

Evenness of the pollinators among the sites was assessed using the Pielou's (*J′*) (Pielou, 1969).

$$J' = \frac{H'}{H_{max}} = \frac{H'}{l_n S}$$

Where, *H* = Information content of a sample

Pi = Proportion of total sample belonging to i^{th} species

S = Total number of species in habitat

3.1.3.3. Relative abundance

Relative abundance (P_i) was calculated using the formula (Achacoso *et al*., 2016),

$$P_i = \frac{n_i}{N} \times 100$$

Where, n_i = Number of individuals of i^{th} species

N= Total number of individuals in all species

3.1.4. Morphological identification of solitary pollen bees

Solitary pollen bee specimens were properly packed and sent for morphological identification to Dr. Amala Udayakumar, Scientist (Entomology), Division of Germplasm Conservation and Utilisation, ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. The specimens which were unidentified from NBAIR were again sent to Dr. Jobiraj T., Assistant Professor, Government College Kodenchery, Kozhikode. The hymenopteran specimens other than solitary bees were sent to Dr. P. Girish Kumar, Scientist C, Zoological Survey of India, Western Ghat Research Center, Kerala.

3.1.5. Molecular characterization of solitary pollen bees

3.1.5.1. Isolation of genomic DNA from solitary pollen bees

Solitary pollen bee specimens collected during the roving surveys were preserved in Eppendorf tubes containing 90 per cent ethyl alcohol and stored in a deep freezer at -20°C in the Molecular laboratory of All India Network Project on Agricultural Ornithology (AINPAO), Vellanikkara. The solitary bee specimens were carefully taken out from the stored vials for the isolation of genomic DNA. The DNA isolation was carried out using the DNeasy blood and tissue kit (Qiagen) method.

3.1.5.1.1. DNA extraction using DNeasy blood and tissue kit method

- A solitary bee specimen was carefully taken out using forceps and gently placed in a fresh autoclaved Eppendorf tube. It was then washed with distilled water by gently shaking the tube in a tilting motion. The process was repeated three to four times until the removal of alcohol content from the bee specimen.
- The bee specimen was then transferred to a new Eppendorf tube, crushed and ground using a sterile micro pestle.
- After that 180 µl of buffer ATL and 20 µl proteinase K were added into the freshly ground specimen and vortexed for 15 sec.
- The slurry was incubated at 56°C for about 1h with gentle mixing of the slurry every 10 min during incubation.
- After the incubation, 200 µl of buffer AL was added to the mixture and vortexed for 15 sec. Then 200 µl of absolute ethanol was added to the mixture and mixed well.

- The supernatant was decanted into a DNeasy mini spin column placed above a 2 ml collection tube and centrifuged (TARSONS Spin Win MC 03 version) at 8,000 rpm for 1 min.
- The flow through in the collection tube was discarded and placed on a new collection tube and 500 µl of buffer AW1 was added to it. The mixture in the mini spin column along with the collection tube was centrifuged at 8,000 rpm for 1 min.
- The flow-through was discarded and the mini spin column was placed on a new collection tube. To that, 500 µl of buffer AW2 was added and centrifuged at 14,000 rpm for 3 min.
- The mini spin column was then placed on a microcentrifuge tube of 1.5 ml and 200 µl of buffer AE was added to elute the DNA into the collection tube. The mixture was centrifuged at 8,000 rpm for 1 min.
- The eluted DNA in the micro-centrifuge tube was then transferred into a new Eppendorf tube, labelled and kept at -20°C deep freezer.

3.1.5.2. NanoDrop spectrophotometry

The purity of DNA was checked using a NanoDrop spectrophotometer (model- JENWAY Genova Nano 737 501, ver. 1.55.3). Nucleic acid shows absorption maxima at 260 nm whereas proteins show peak absorbance at 280 nm. Absorbance was recorded at both wavelengths and the purity was indicated by the ratio OD_{260}/OD_{280}. A value between 1.8 and 2.0 indicated that the DNA was pure and free from proteins and RNA.

3.1.5.3. Amplification of mitochondrial cytochrome oxidase I gene (mtCOI)

Polymerase chain reaction (PCR) was carried out for the amplification of mtCOI genes in the PCR Invitrogen Veriti Thermal cycler (Applied Biosystems) using specific primers (Table 1).

Table 1. Details of primers used for amplification of mtCOI locus of solitary pollen bees

Primer Details	5' - 3'	Reference
Forward primer- LCO1490	GGTCAACAAATCATAAAGATATTGG	Magnacca and Brown, 2012
Reverse primer- HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	

3.1.5.4. Standardisation of annealing temperature for the primers

The PCR reaction was set up in varying temperatures to standardize the annealing temperature using PCR Invitrogen Veriti Thermal cycler (Applied Biosystems). The temperatures used for standardization were 50°C, 52°C, 53°C, 54°C, 55°C, 56°C, 58°C and 60°C. The best temperature was selected based on the quality of DNA band obtained in the gel electrophoresis. The composition of PCR reaction mixture is given in Table 2.

Table 2. PCR reaction mixture composition for solitary pollen bees

Component	Quantity (µl)/reaction
Template DNA (50 ng/µl)	2
PCR mastermix	10
Forward primer	0.6
Reverse primer	0.6
Distilled water	6.8
Total	20

The template DNA, forward and reverse primers, PCR master mix (TAKARA EmeraldAmp®), Milliporewater® were mixed thoroughly with the help of mini spinner (TARSONS SPINWIN MC-00) and were placed immediately into the thermal cycler. PCR reaction was run at the best found temperature during the standardisation of DNA. Details of PCR reaction programme are given in Table 3.

Table 3. PCR programme for solitary pollen bees

Steps	Temperature (°C)	Time	
Initial denaturation	94	2 min	
Denaturation	94	30 sec	35 cycles
Annealing	52	1 min	
Extension	72	45 sec	
Final extension	72	10 min	

3.1.5.5. Gel documentation of the PCR products

3.1.5.5.1. Agarose gel electrophoresis

The PCR product was subjected to agarose gel electrophoresis to confirm the amplification of the mtCOI region.

- The gel casting tray was wiped with the help of ethyl alcohol (70 %) to remove any foreign particles and was allowed to dry well
- 1X TAE buffer was prepared by diluting 50X TAE stock solution i.e., 1 ml of 50X TAE buffer was added to 49 ml distilled water
- Agarose 1.2 g was weighed and transferred into a microwave-safe conical flask containing 100 ml of 1X TAE buffer and mixed well
- The solution was heated in a microwave for 30 seconds till the agarose got completely dissolved
- The agarose solution was kept at room temperature and cooled up to 45°C
- Ethidium Bromide (EtBr) was added (3µl) to the solution and mixed well
- The mixture was poured into the gel casting tray with the combs in place
- The mixture was kept in the gel casting tray for about 30 min to get it solidified

3.1.5.5.2. Loading samples and running agarose gel electrophoresis of PCR product

- Once solidified the agarose gel was transferred into an electrophoresis tank with 1X TAE buffer such that the wells were at the cathode side

(make sure that the wells in the gel got completely submerged in the buffer).

- DNA ladder (Thermo Scientific ^{TM}), 100 bp (3 µl) was loaded carefully into the first well using a micropipette to determine the size of the PCR product.
- PCR product (5 µl) was carefully loaded into the wells using a micropipette
- The gel was run at 60 volts until the dye had approximately covered three a fourth of the gel.
- The device was turned off and disconnected from the electrodes and the gel was removed from the tank.
- Visualization of the amplified product and image capturing of the gel was done using a gel documentation unit (Invitrogen Life Technologies E-Gel imager).

3.1.5.6. DNA sequencing of the product

The amplified PCR product was sent to AgriGenome Labs Private Limited, Cochin and Biokart India Private Limited, Bengaluru for sequencing using forward and reverse primers.

3.1.5.7. Data analysis using *In-silico* tools

3.1.5.7.1. Sequence analysis

The forward and reverse sequences of each species had been assembled using the CAP3 sequence assembly program (Huang and Madan, 1999) to develop contigs. Mega 7 software was used to analyse the existence of stop codons in the developed contigs.

3.1.5.7.2. Sequence homology analysis

The sequence homology of the contigs of each solitary bee specimen was determined using the search tool by National Center for Biotechnology Information (NCBI) called BLASTn (Basic Local Alignment Search Tool for nucleotides). The query sequences were uploaded to NCBI BLASTn to get similar sequences from NCBI. The programme showed already deposited subject sequences which had similarities with the query sequence and also provided additional information such as

per cent sequence identity, E value and query cover. Species determination was carried out using similar sequences from NCBI.

3.1.5.8. Barcoding of solitary pollen bees

The sequences generated using the DNA barcode primer and specimen details from Sanger sequencing were submitted to the Barcode of Life Data System (BOLD system v4). An account was opened in the workbench session of the BOLD system v4 database and a new project was created. Specimen data *viz*., specimen identifiers, specimen taxonomy, and specimen collection details were submitted and an auto-generated process ID was obtained. Further, the primer details, high-resolution specimen images, mitochondrial DNA sequences (in fasta format) and trace files (in .ab1 format) obtained from the sequencer were uploaded to the database and the corresponding barcode of a particular specimen was generated.

3.2. Determination of the peak foraging time of solitary pollen bees

Two different cucurbit crops *viz*., bitter gourd (*Momordica charantia* L.) and oriental pickling melon (*Cucumis melo* var. *conomon* Mak.) were raised separately at post-monsoon 2018, summer 2019 and monsoon 2019 to study the foraging behaviour of the most frequent bee pollinators. Pollinators were observed for the collection of nectar and pollen, and the number of bee visits per square meter area to record their peak foraging time.

3.2.1. Temporal variations of different insect visitors

The foraging activity of bee pollinators was observed to study the temporal variations in their foraging behavior. For this, the total number of bees visiting the gourd flowers was recorded in a square meter area for five minutes from 06.00 h to 12.00 h starting from their 50 per cent blooming period. Ten such observations were made at hourly intervals. The observations were taken for 100 days and the mean number of solitary pollen bees per square meter area was calculated to find out the most dominant solitary bee pollinator of gourd flowers. The abundance of major flower visitors was recorded at 100 per cent flowering of gourd flowers for 5 days. The number of visits made by major flower visitors at 25, 50, 75 and 90 per cent flowering was also recorded for 5 days. Descriptive statistics were done to analyse the data using SPSS 21 software.

3.3. Studying the nesting preferences of solitary pollen bees

3.3.1. Artificial nesting sites for attracting solitary bees

Artificial nesting cavities or trap nests were prepared to attract the cavity nesting solitary pollen bees. Artificial nests of varied nest hole sizes *viz*., 2-4, 4.1-.6, 6.1-9, 9.1-12, and 12.1-15 mm with locally available materials like wood and tubular plant materials were placed at specific distances *viz*., 10, 100 and 250 m away from the field to attract various species of solitary pollen bees. The suitability of the nests was assessed based on the per cent occupation to the nest by the solitary bee species. The number of nests occupied by the solitary bees were recorded at monthly interval. Nesting sites occupied by other arthropods were also recorded to know the preference and competition for trap nests. For these, observations were also made on the per cent occupancy by the non-solitary bee arthropods across the artificial nesting cavities. The nesting site competition was analysed using the per cent nest occupancy between the solitary bees and non-solitary bee arthropods. The data were subjected to a three-factor factorial analysis using the statistical software GRAPES 1.1.0.

3.3.2. Nesting architecture and biology of major solitary bee pollinators of selected cucurbit crops

The nesting architecture and biology of major solitary bee pollinators such as the small carpenter bees *viz*., *Ceratina hieroglyphica* and *Ceratina binghami* and the Allodapine bee *Braunsapis picitarsis* were studied from October 2019 to January 2021. For these, nests of the small carpenter bees and allodapine bees were searched intensively in KAU, Vellanikkara campus. Nests were located by tracking the foraging bees and once the nesting site was located, the area was searched for more nests.

3.3.2.1. Nesting architecture

A total of 199 nests of small carpenter bees and 83 nests of allodapine bees were collected during the study period to observe the general nesting architecture of bees. Individual nests were dissected carefully with a sharp blade to give a gentle split lengthwise and classified into five categories (Daly, 1966) *viz.*, hibernacula nests, founding nests, active brood nests, full brood nests and mature brood nests according to the life stages of bees and conditions of nests constructed by the bees. Hibernacula nests include nests with remnants of previously built nest cells, darkened walls and fecal remains, pollen residues, or molted skins from the previous breeding season as well as the presence or absence of adult bees in them. Founding nests are those with

adult bees that are actively working on the construction of new cells and are devoid of immature stages and pollen-masses. Active brood nests always contain pollen masses in each constructed cell with freshly laid eggs or immature stages in them. Full brood nests are those which contain various immature stages of bees with different proportion of pollen masses that shows active feeding of pollen by the larva. Mature brood nests include the nests inhabited by an adult bee with their callow offsprings, where the mother bee interact with young offsprings of both sexes.

The nest architecture of each species such as entrance diameter, the thickness of nesting stem, occupied nest length, individual brood cell length, cell septum thickness, number of cells per nest, number of immature stages per nest, the weight of pollen provision per brood cell and number of adults in the nest during collection were recorded.

3.3.2.2. Nesting biology

The nesting biology of small carpenter bees and allodapine bees was also studied (N=30 nests). For these, the immature stages of bees collected from the nests were reared at the laboratory (28±2 °C and 75±1 % RH), where the split stems were tied properly with rubber bands and kept in rearing boxes with proper aeration. The stems were opened daily to observe the developmental duration of different life stages. A cotton swab soaked in 10 per cent honey solution was kept in rearing boxes and the adult longevity was also recorded. Descriptive statistics and two sample t-test was used to analyze the data with the software SPSS 21.

3.4. Study on palynology of solitary pollen bees

Palynological studies were conducted to observe the diversity of pollen collected by solitary pollen bees. For these, pollen grains were collected from either pollinator's body or their natural and artificial nesting sites using a fine-sterilized camel brush. The forceps were used to capture the solitary bee species to collect the pollen from their body surface and the collected pollen grains were immediately transferred into Eppendorf tubes containing 70 per cent ethyl alcohol with proper labels.

3.4.1. Preparation of pollen reference slides

Major flora in the study area was recorded at monthly intervals and reference pollen slides were prepared from fresh pollen samples collected from freshly opened flowers.

3.4.2. Identification of pollen samples using light microscopy

Pollen samples collected from the body surface of solitary pollen bees and their nesting sites were stored in 70 per cent alcohol. From this, 5 µl of pollen sample was carefully taken out using a micropipette and added into a microcentrifuge tube. The pollen sample (1 ml) was subjected to centrifugation using a microcentrifuge (TARSONS SPINWIN MC 03 version) at 3,000 rpm for 3 minutes. The supernatant was decanted and 1 ml of sterile distilled water was added to the microcentrifuge tube to properly wash and hydrate the pollen sample. The pollen sample immersed in sterile distilled water was subjected to centrifugation at 3,000 rpm for 3 minutes. The process was repeated three to four times and 10 µl of pollen sample was taken using a micropipette and placed on a microscopic slide to view under the microscope. The general morphological characteristics of pollen were observed using an Olympus® CX43 Trinocular Microscope at AICRP on BCCP laboratory, KAU, Vellanikkara.

3.4.3. Identification of pollen samples using Scanning Electron Microscope (SEM)

3.4.3.1. Processing of pollen samples for sputter coating

The pollen samples which were stored in 70 per cent ethyl alcohol were used for SEM photography. For this, a pollen sample (1 ml) was taken using a micropipette and added into a fresh sterilized microcentrifuge tube (2 ml capacity). The sample was then subjected to centrifugation using a microcentrifuge (TARSONS SPINWIN MC 03 version) at 3,000 rpm for 3 minutes. The supernatant was decanted and 2 ml of 50 per cent ethanol was added to the pollen pellet. The pollen sample was centrifuged at 3,000 rpm for 10 min and the supernatant was again decanted. Likewise, each pollen sample was subjected to dehydration through an ascending ethanolic series of 70, 90 and 100 per cent, each at 3,000 rpm for 10 min. The dehydrated pollen samples stored in absolute alcohol were later used for sputter coating.

For sputter coating, 10 µl of pollen sample was pipetted out using a micropipette and smeared on a carbon tape which was placed on a sample stub.

3.4.3.2. Sputter coating of pollen samples

- The sputter coater was turned on and the nitrogen cylinder was set at 1 bar.
- As the sputter coater chamber was always under vacuum, it was opened by pressing on the vent knob to bring the sputter coater chamber into atmospheric pressure.
- The sample stubs were carefully placed in the chamber and immediately closed, to prevent the entry of any foreign particle into the chamber.
- The sample was kept in the sample chamber until the vacuum reached 8×10^{-2} mBar.
- The plasma push button was pressed and held for 30 sec. The leak knob was slowly rotated in an anticlockwise direction till the current reached 10mA, where the time was set up to 60 sec.
- The plasma push button was released and allowed to stabilize for 30 sec.
- The sputter coating was initiated by pressing the start button and the leak knob was closed once the process was over by turning the leak knob in a clockwise direction.
- The sputter coater chamber was opened and the samples were carefully taken using forceps and placed on the micro tip holder.

3.4.3.3. SEM imaging of pollen samples

SEM imaging of samples that were sputter coated with gold particles was carried out using a Scanning Electron Microscope (TESCAN Vega-3-LMU) at Central Instruments Laboratory, KVASU, Thrissur. General characteristics and dimensions of pollen were recorded accordingly.

3.4.4. Morphological characterization and identification of pollen grains

Pollen grains subjected to light microscopy and scanning electron microscopy were morphologically described by several palynological terminologies (Halbritter *et al*., 2018) and identified using the previously prepared reference pollen slides. Every pollen image was cross-checked for morphological identity with the data obtained from the palynological database *i.e*., PalDat 3.4 version.

PalDat 3.4, is the world's largest database for palynological data, which has information on a large variety of pollen grains in the world. The data on pollen grains which were obtained during the palynological studies of solitary pollen bees were

later uploaded in PalDat with proper descriptions. Terminologies used for the morphological description of pollen grains are mentioned as follows;

- Pollen dispersal unit: Monad and tetrad
- Size of pollen: Small (10-24 µm), medium (25-49 µm) and large (50-99 µm) based on its diameter
- Shape: Circular, prolate, spheroid, triangular, *etc* based on their polar and equatorial axes
- Aperture: Inaperturate, pantocolpate, tricolpate, spiraperturate, *etc.*
- Ornamentation: Psilate, reticulate, echinate, verrucate, microrugulate, *etc.*

3.4.5. Estimation of pollen grains

The number of loose pollen grains sticking onto the body surface of major bee pollinators of selected cucurbit crops such as bitter gourd and oriental pickling melon was estimated using a Neubauer hemocytometer. The foraging bees were captured gently and transferred into vials kept in cooling racks and brought to the laboratory. The bees were shaken gently and all the loose pollen grains were collected and placed in Eppendorf tubes containing 70 per cent alcohol. After that, 1 ml of pollen sample was taken and added into a new Eppendorf tube and 4 ml of distilled water was added to it. Then 10 µl of the sample was taken out using a micropipette and released into the hemocytometer. The number of pollen grains in 4 corner squares of the hemocytometer was counted and the total number of pollen grains in the sample was estimated using the formula,

Number of pollen grains/ml = Average pollen grains counted per square × Dilution factor $\times 10^4$

3.5. Determination of the effect of different plant protection measures on pollination in selected cucurbitaceous crops

The field experiment was conducted to assess the impact of commonly used plant protection measures on pollination. Two crops *viz*., bitter gourd and oriental pickling melon were raised separately and the activity of pollinating bees was observed before and after the application of plant protection measures. Observation on yield parameters was studied by selective exclusion of pollinating bees as control and compared with the yield of open-pollinated plants.

The seeds of bitter gourd (var: *Preethi*) and oriental pickling melon (var: *Saubhagya*) were procured from Central Nursery, KAU, Vellanikkara. The fields were raised in 2.1 cents for each crop having a spacing of 2.0×2.0 m for bitter gourd and 2.0×1.5 m for oriental pickling melon at three different seasons *viz*., post-monsoon-2018, summer-2019 and monsoon-2019. Two control treatments were maintained having three replications for each, where one control treatment was caged to observe the yield difference and the other was kept for open pollination by bees. The control treatment with open pollination was taken as the major control for assessment of the effect of plant protection measures (7T × 3R) on pollination to the gourd flowers (Table 4).

Table 4. Treatments for assessing the effect of plant protection measures on bee visit

Sl. No.	Treatments
T_1	Dimethoate 30 % EC (@ 300 g ai ha^{-1})
T_2	Imidacloprid 200 SL (@ 30 g ai ha^{-1})
T_3	Azadirachtin 300 ppm (@ 0.03 %)
T_4	*Beauveria bassiana* (@ 1×10^8 spores ml^{-1})
T_5	Mancozeb 75 WP (@ 0.15%)
T_6	Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2%)
T_7	Untreated control

The number of bee visits before and after the application of plant protection measures was recorded. The fruit set in open-pollinated plants and caged plants were recorded. The mean number of bee visits before and after the application of treatments were analysed by Analysis of Variance (ANOVA). Reduction in the number of bee visits was analysed by ANOVA and means were separated by Duncan's Multiple Range Test (DMRT).

Results

4. RESULTS

Results of the investigation on "Pollination ecology of solitary pollen bees" carried out at the Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellanikkara are presented here.

4.1 Documentation of pollinator diversity in selected cucurbitaceous crops

The insect pollinator diversity and their abundance in two cucurbitaceous crops *viz.,* bitter gourd and oriental pickling melon were recorded by roving survey in central districts of Kerala *i.e*., Thrissur, Palakkad and Ernakulam from March 2018 to December 2019 (Table 5).

Insect visitors documented from bitter gourd and oriental pickling melon revealed a total of 45 insect species belonging to 11 families and three orders. The hymenopterans were the major flower visitors which comprised of 41 species *viz*., *Braunsapis picitarsis* (Cameron), *Braunsapis mixta* (Smith), *Ceratina smaragdula* (F.), *Ceratina binghami* Cockerell, *Ceratina hieroglyphica* Smith, *Ceratina* sp*., Xylocopa ruficornis* Fab., *Xylocopa fenestrata* Fab., *Amegilla zonata* (L.), *Thyreus* sp. 1, *Thyreus* sp. 2, *Apis cerana* Fab., *Apis florea* Fab., *Apis dorsata* Fab., *Tetragonula iridipennis* Smith., *Halictus* sp., *Lasioglossum serenum* (Cameron), *Nomia curvipes* (Fab.), *Hoplonomia elliotti* (Smith), *Gnathonomia thoracica* Smith, *Leuconomia interstitialis* Cameron, *Nomiapis* sp*., Lipotriches* sp., *Coelioxys* sp., *Megachile disjuncta* (Fab.), *Megachile* sp. 1, *Megachile* sp. 2, *Antodynerus punctatipennis* (de Saussure), *Delta pyriforme* (Fabricius), *Eumenes macrops* de Saussure, *Phimenes flavopictus* (Blanchard), *Rhynchium brunneum* (Fabricius), *Ropalidia brevita* Das & Gupta, *Phalerimeris phalerata turneri* (Betrem), *Scolia affinis* (Guerin), *Scolia cyanipennis* Fabricius, *Chalybion bengalense* (Dahlbom), *Sphex argentatus* Fabricius*, Sphex sericeus* (Fabricius), *Chrysis* sp. and *Tachytes* sp. There were four other flower visitors documented from selected cucurbitaceous crops *viz.*, *Chrysomya* sp. and *Syritta* sp. which belonged to the order Diptera and *Eurema* sp. and *Acytolepis* sp. which belonged to the order Lepidoptera (Table 6) (Plate 1a - 1S).

Table 5. Details of locations and host plants recorded during the roving survey 2018-2019

District	Places	GPS coordinates	Host plants	Survey code	Sample code
Thrissur	Elanad	10.6275 & 76.3955	Bitter gourd (*Momordica charantia*), touch-me-not (*Mimosa pudica*), ash gourd (*Benincasa hispida*), snake gourd (*Trichosanthes anguina*), oriental pickling melon (*Cucumis melo*), pumpkin (*Cucurbita moschata*), Singapore daisy (*Spagneticola trilobata*), little tree plant (*Biophytum sensitivum*)	El-2018Apr, El-2018Jun, El-2018Aug, El-2018Oct, El-2019Jan, El-2019Mar, El-2019Apr, El-2019jun, El-2019Aug	SPB1, SPB2, PB13, PB26, PB1, PB12,
	Cherumkuzhy	10.5151 & 76.3239	Bitter gourd (*Momordica charantia*),	Cz-2019Nov	SPB1, PB13, PB 26, PB14,
	Punnayurkulam	10.6855 & 76.0114	Bitter gourd (*Momordica charantia*), Siam weed (*Chromoleana odorata*), touch-me-not (*Mimosa pudica),* little tree plant (*Biophytum sensitivum*), Singapore Daisy (*Spagneticola trilobata*), butterfly-pea (*Clitoria ternatea*)	Py-2019Jan	PB3, PB12, SPB1, SPB2, PB23, PB30
	Vellanikkara	10.5452 & 76.2739	Bitter gourd (*Momordica charantia*), touch-me-not (*Mimosa pudica*), ash gourd (*Benincasa hispida*), snake gourd (*Trichosanthes anguina*), oriental pickling melon (*Cucumis melo*), Pumpkin (*Cucurbita moschata*), little tree plant (*Biophytum sensitivum*), Singapore daisy (*Spagneticola trilobata*), butterfly-pea (*Clitoria ternatea*),	Vka-2018Jan, 2018Mar, 2018Apr, 2018May, 2018Jun, 2018Oct, 2018Nov, 2018Dec	SPB1, SPB2, PB1, PB3, PB6, PB8, PB10, PB12, PB13, PB15, PBAD1, PB26, PB24

			peacock-flower-tree (*Caesalpinia pulcherrima*), thumba (*Leucas aspera*), basil holy(*Ocimum sanctum*), patchouli (*Pogostemon* sp.)		
	Mannamangalam	10.4878 & 76.3434	Bitter gourd (*Momordica charantia*)	Mn-2019oct	PB1, PB12
	Eravimangalam	10.9444 & 76.2435	Bitter gourd (*Momordica charantia*), Oriental pickling melon (*Cucumis melo*)	Er-2019Nov	
	Peechi	10.5437 & 76.2749	Bitter gourd (*Momordica charantia*), oriental pickling melon (*Cucumis melo*)	Pc-2018Feb, Pc-2018Mar, Pc-2018Aprl	SPB1, PB1, PB12
	Nadathara	10.5437 & 76.2749	Bitter gourd (*Momordica charantia*), oriental pickling melon (*Cucumis melo*) touch-me-not (*Mimosa pudica*)	Nd-2018Jun, Nd-2018Oct, Nd-2018Nov	PB19, PB13, SPB1, SPB2
	Pazhayannur	10.6823 & 76.4230	Bitter gourd (*Momordica charantia*), touch-me-not (*Mimosa pudica*), ash gourd (*Benincasa hispida*), snake gourd (*Trichosanthes anguina*), little tree plant (*Biophytum sensitivum*), Singapore daisy (*Spagneticola trilobata*)	Pz-2018Jan, Pz-2018Jun, Pz-2018Aug, Pz-2018Nov	SPB1, SPB2
	Kattilapoovam	10.5988 & 76.2994	Bitter gourd (*Momordica charantia*), yellow cosmos (*Cosmos sulphureus*), little tree plant (*Biophytum sensitivum*)	KTP-2018Oct	SPB1, SPB2
	Thanikkudam	10.5722 & 76.2624	Bitter gourd (*Momordica charantia*), snake gourd (*Trichosanthes anguina*), oriental pickling melon (*Cucumis melo*), pumpkin (*Cucurbita moschata*)	THK-2018Sep	SPB1, SPB2, PB1, PB14
	Ayyanthole	10.5245 & 76.1941	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Singapore daisy (*Sphagneticola trilobata*)	AYY-2018Oct	SPB1

	Chazhur	10.4351 & 76.1407	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*),	CHZ-2018Oct	SPB1,PB1, PB12
	Varanthirappilly	10.4276 & 76.3334	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*)	Vr-2018Dec	SPB1,PB1
	Chalakkudy	10.5348 & 76.1824	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*),	Ck-2018Oct	PB12,PB14
Palakkad	Alathur	10.6454 & 76.5458	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Snake gourd (*Trichosanthes anguina*)	Al-2018Jun, Al-2018Nov, Al-2018Dec	PB1,PB12
	Nenmara	10.5934 & 76.6005	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*),	Nm-2018Feb	SPB1,SPB2
	Manjaloor	10.6697 & 76.6063	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*)	Mj-2018Mar, Mj-2019oct	SPB1
	Kottopadam	10.9980 & 76.3912	Bitter gourd (*Momordica charantia*), Oriental pickling melon (*Cucumis melo*), Pumpkin (*Cucurbita moschata*), Touch-me-not (*Mimosa pudica*), Little tree plant (*Biophytum sensitivum*),	Kt-2018Nov Kt-2018Dec	PB1, PB25, SPB1, PB16
	Mundur	10.8241 & 76.5886	Bitter gourd (*Momordica charantia*), Pumpkin (*Cucurbita moschata*), Touch-me-not (*Mimosa pudica*), Singapore Daisy (*Sphagneticola trilobata*), Snake gourd (*Trichosanthes anguina*)	Md-2019Jan	SPB2, PB29, PB17, PB12
	Anavari	10.4022 & 76.3510	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Ivy gourd (*Coccinia grandis*), Tridax daisy (*Tridax procumbens*)	An-2019Oct	PB1, PB12
	Manjavari	10.3953 & 76. 3321	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Ivy gourd	Mi-2019Oct	PB1, PB12, Pb26

			(*Coccinia grandis*), Little ironweed (*Cyanthillium synereum*)		
Ernakulam	Kodanad	10.1146 & 76.4777	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Singapore Daisy (*Spagneticola trilobata*), Peacock-flower tree (*Caesalpinia pulcherrima*), Basil (*Ocimum tenuiflorum*), Little tree plant (*Biophytum sensitivum),* Joyweed *(Alteranthera* sp.*)*	Kd-2019Nov	SPB1, SPB2, PB1, PB12
	Malayattur	10.1955 & 76.4968	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Singapore Daisy (*Spagneticola trilobata),* Jungle geraneum *(Ixora coccinia)*	Ml-2019Nov	PB1, PB12, PB14, PB27
	Cheranallur	10.0615 & 76.2886	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Singapore Daisy (*Spagneticola trilobata*	Cl-2019Nov	SPB1, PB1, PB12, PB22
	Edavoor	10.1702 & 76.4643	Bitter gourd (*Momordica charantia*), Touch-me-not (*Mimosa pudica*), Singapore Daisy (*Spagneticola trilobata)*	Ed-2019Nov	PB1, PB12
	Kalady	10.1710 & 76.4468	Bitter gourd (*Momordica charantia),* Singapore Daisy (*Spagneticola trilobata),*	Ka-2019Nov	PB13, SPB2

Table 6. Pollinator diversity recorded in the selected cucurbitaceous crops during roving survey 2018-2019

Order	Family	Species	Reward
Hymenoptera	Apidae	*Braunsapis picitarsis*	Nectar+Pollen
		Braunsapis mixta	Nectar+Pollen
		Ceratina smaragdula	Nectar+Pollen
		Ceratina binghami	Nectar+Pollen
		Ceratina hieroglyphica	Nectar+Pollen
		Ceratina sp.	Nectar+Pollen
		Xylocopa ruficornis	Nectar
		Xylocopa fenestrata	Nectar
		Amegilla zonata	Nectar
		Thyreus sp. 1	Nectar
		Thyreus sp. 2	Nectar
		Apis cerana	Nectar+Pollen
		Apis florea	Nectar+Pollen
		Apis dorsata	Nectar
		Tetragonula iridipennis	Nectar+Pollen
	Halictidae	*Halictus* sp.	Nectar+Pollen
		Lasioglossum serenum	Nectar+Pollen
		Nomia curvipes	Nectar+Pollen
		Hoplonomia elliotti	Nectar+Pollen
		Gnathonomia thoracica	Nectar+Pollen
		Leuconomia interstitialis	Nectar+Pollen
		Nomiapis sp.	Nectar+Pollen
		Lipotriches sp.	Nectar+Pollen
	Megachilidae	*Coelioxys* sp.	Nectar
		Megachile disjuncta	Nectar+Pollen
		Megachile sp. 1	Nectar+Pollen
		Megachile sp. 2	Nectar+Pollen
	Vespidae	*Antodynerus punctatipennis*	Nectar
		Delta pyriforme	Nectar

		Eumenes macrops	Nectar
		Phimenes flavopictus	Nectar
		Rhynchium brunneum	Nectar
		Ropalidia brevita	Nectar
	Scoliidae	*Phalerimeris phalerata turneri*	Pollen
		Scolia affinis	Pollen
		Scolia cyanipennis	Pollen
	Sphecidae	*Chalybion bengalense*	Pollen
		Sphex argentatus	Pollen
		Sphex sericeus	Pollen
	Chrysididae	*Chrysis* sp.	Nectar
	Crabronidae	*Tachytes* sp.	Pollen
Diptera	Calliphoridae	*Chrysomya* sp.	Pollen
	Syrphidae	*Syritta* sp.	Pollen
Lepidoptera	Pieridae	*Eurema* sp.	Nectar
	Lycaenidae	*Acytolepis* sp.	Nectar

4.1.1. Total pollinator abundance in three districts of central Kerala

The per cent abundance of flower visitors documented from the three central districts of Kerala was calculated. Among all the insect visitors, 41 species were Hymenopterans (91.11 %) followed by 2 species from Diptera (4.44 %), and the other 2 species belonged to Lepidoptera (4.44 %). The order Hymenoptera was represented by 41 species under which, 15 species of Apidae (36.58 %), 8 species of Halictidae (19.51%), 4 species of Megachilidae (9.76 %), 6 species of Vespidae (14.63 %), 3 species of Scoliidae (7.32%), 3 species of Sphecidae (7.32 %), 1 species of Chrysididae (2.44 %) and 1 species of Crabronidae (2.44 %) were included. Among the flower visitors, non-*Apis* bees (62.67 %) were the most abundant as compared to *Apis* bees (37.32 %) in the sweep net collection. The non-*Apis* bees comprised of stingless bees (30.49 %), allodapine bees (11.20 %), small carpenter bees (37.34 %), large carpenter bees (0.83 %), blue banded bees (1.45 %), cuckoo bees (0.62 %), sweat bees (15.35 %) and megachilid bees (2.69 %).

The relative species abundance of all pollinators was calculated by dividing the number of each species by the total number of recorded species during the roving survey (Table 7). The abundance of different insect families revealed that Apidae (83.70 %) was the most dominant family followed by Halictidae (9.05 %), Vespidae (1.94 %), Megachilidae (1.46 %), Crabronidae (0.73 %), Sphecidae (0.72 %), Syrphidae (0.49 %), Scoliidae (0.48 %), Pieridae (0.36 %), Chrysididae (0.24 %), Calliphoridae (0.24 %) and Lycaenidae (0.12 %).

Of the total insect visitors of bitter gourd and oriental pickling melon flowers collected through the sweep net from all three districts, *T. iridipennis* (18.05 %) was found to be the predominant species followed by *A. cerana* (17.07 %), *A. florea* (15.11 %), *C. hieroglyphica* (11.54 %), *C. smaragdula* (9.82 %) and *B. picitarsis* (6.01 %) (Table 3). Relative abundance of other bee species was found between 0.12 per cent to 3.07 per cent. The insect visitors with the lowest relative bundance were, *L. interstitialis, Nomiapis* sp., *Lipotriches* sp., *Coelioxys* sp., *Megachile* sp. 1, *D. pyriforme, E. macrops, P. flavopictus, P. phalerata, S. affinis, S. argentatus* and *Acytolepis* sp.

Table 7. Relative abundance of insect visitors documented during the roving survey

Order	Family	Species	Relative Species abundance (%)	Total abundance (%)
Hymenoptera	Apidae	*Braunsapis picitarsis*	6.01	83.70
		Braunsapis mixta	0.61	
		Ceratina smaragdula	9.82	
		Ceratina binghami	0.49	
		Ceratina hieroglyphica	11.54	
		Ceratina sp.	0.24	
		Xylocopa ruficornis	0.24	
		Xylocopa aestuans	0.24	
		Amegilla zonata	0.85	
		Thyreus sp. *1*	0.24	
		Thyreus sp. *2*	0.12	
		Apis cerana	17.07	
		Apis florea	15.11	
		Apis dorsata	3.07	

		Tetragonula iridipennis	18.05	
	Halictidae	*Halictus* sp.	2.21	9.05
		Lasioglossum serenum	0.85	
		Nomia curvipes	5.15	
		Hoplonomia elliotti	0.24	
		Gnathonomia thoracica	0.24	
		Leuconomia interstitialis	0.12	
		Nomiapis sp.	0.12	
		Lipotriches sp.	0.12	
	Megachilidae	*Coelioxys* sp.	0.12	1.46
		Megachile disjuncta	0.98	
		Megachile sp. *1*	0.12	
		Megachile sp. *2*	0.24	
	Vespidae	*Antodynerus punctatipennis*	0.49	1.94
		Delta pyriforme	0.12	
		Eumenes macrops	0.12	
		Phimenes flavopictus	0.12	
		Rhynchium brunneum	0.85	
		Ropalidia brevita	0.24	
	Scoliidae	*Phalerimeris phalerata*	0.12	0.48
		Scolia affinis	0.12	
		Scolia cyanipennis	0.24	
	Sphecidae	*Chalybion bengalense*	0.36	0.72
		Sphex argentatus	0.12	
		Sphex sericeus	0.24	
	Chrysididae	*Chrysis* sp.	0.24	0.24
	Crabronidae	*Tachytes* sp.	0.73	0.73
Diptera	Calliphoridae	*Chrysomya* sp.	0.24	0.24
	Syrphidae	*Syritta* sp.	0.49	0.49
Lepidoptera	Pieridae	*Eurema hecabe*	0.36	0.36
	Lycaenidae	*Acytolepis* sp.	0.12	0.12

4.1.2. Comparison of diversity indices of all pollinators among the three districts of Central Kerala

Diversity indices were used to study the diversity, richness, and evenness of the pollinators collected from three districts of Central Kerala (Table 8). The diversity of various insect species collected was measured by Simpson's diversity index (1-D), Shannon-Weiner index (H'), Brillouin index (H_B), and Berger-Parker index. Whereas, the species richness of each district was measured by Menhinick and Margalef's indices. The evenness of the species distributed in the surveyed area was calculated by Pielou's evenness (J) index.

The diversity study revealed that the Thrissur district has a high number of taxa (33) followed by Palakkad (29) and Ernakulam (17) districts. The total number of individuals in the sweep net collection was also high in Thrissur district (416) as compared to Palakkad (267) and Ernakulam (131) districts. Simpson's Dominance (D) was found to be high in Ernakulam district (0.17) followed by Palakkad (0.14) and Thrissur (0.09), which clearly showed that the diversity of species is low in Ernakulam district as compared to that of the other two districts. This was confirmed with the Simpson's diversity index (1-D) in which Thrissur district showed the highest value (0.90) followed by Palakkad (0.85) and Ernakulam (0.82) districts. Shannon diversity index (H') for all the three districts ranged from 2.00 to 2.6 which indicated the presence of a moderately diverse species in each district. Among the Shannon index values, the Thrissur district showed the highest value (2.59) which was followed by Palakkad (2.29) and Ernakulam (2.07). Thus, Ernakulam district was considered as the area with low species abundance and evenness compared to the other two districts during the survey. Brillouin index (H_B) was found to be the highest in the Thrissur district (2.47) and the lowest in the Ernakulam district (1.89), which confirmed that the species abundance was very high in Thrissur district. The Berger-Parker index was found higher in the Ernakulam district (0.26) followed by Palakkad (0.23) and Thrissur (0.13) districts, which showed that in the Ernakulam district the pollinator community is dominated by the most common species.

The species richness of the three districts was compared with the help of Menhinick's index and Margalef's index. In the present study, Menhinick's index was high in the Palakkad district (1.77) followed by Thrissur (1.61) and Ernakulam (1.48)

1a.) *Braunsapis picitarsis* (Cameron)

1b.) *Braunsapis mixta* (Smith)

1c.) *Ceratina smaragdula* (F.)

1d.) *Ceratina binghami* Cockerell

1e.) *Ceratina hieroglyphica* Smith

1f.) *Ceratina* sp.

Plate 1 a-1f: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1g.) *Xylocopa ruficornis* Fab.

1h.) *Xylocopa fenestrata* Fab.

1i.) *Amegilla zonata* (L.)

1j.) *Thyreus* sp. 1

1k.) *Thyreus* sp. 2

1l.) *Apis cerana* Fab.

Plate 1g-1l: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1m.) *Apis florea* Fab.

1n.) *Apis dorsata* Fab.

1o.) *Tetragonula iridipennis* Smith.

1p.) *Halictus* sp.

1q.) *Lasioglossum serenum* (Cameron)

1r.) *Nomia curvipes* (Fab.)

Plate 1m-1r: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1s.) *Hoplonomia elliotti* (Smith)

1t.) *Gnathonomia thoracica* Smith

1u.) *Leuconomia interstitialis* Cameron

1v.) *Nomiapis* sp.

1w.) *Lipotriches* sp.

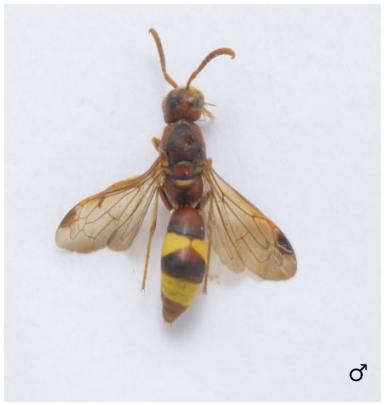
1x.) *Coelioxys* sp.

Plate 1s-1x: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1y.) *Megachile disjuncta* (Fab.)

1z.) *Megachile* sp. 1

1A.) *Megachile* sp. 2



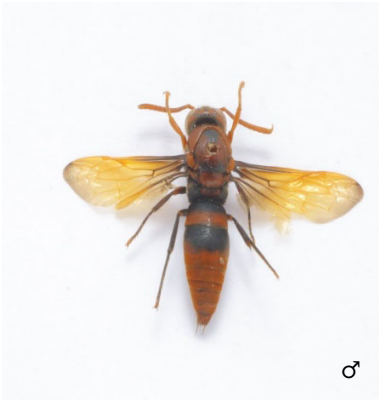
1B.) *Antodynerus punctatipennis* (de Saussure)

1C.) *Delta pyriforme* (Fabricius)

1D.) *Eumenes macrops* de Saussure

Plate 1y-1D: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1E.) *Phimenes flavopictus* (Blanchard)



1F.) *Rhynchium brunneum* (Fabricius)

1G.) *Ropalidia brevita* Das & Gupta

1H.) *Phalerimeris phalerata turneri* (Betrem)

1I.) *Scolia affinis* (Guerin)

1J.) *Scolia cyanipennis* Fabricius

Plate 1E-1J: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1K.) *Chalybion bengalense* (Dahlbom)

1L.) *Sphex argentatus* Fabricius

1M.) *Chrysis* sp.

1N.) *Sphex sericeus* (Fabricius)

1O.) *Tachytes* sp.

1P.) *Syritta* sp.

Plate 1K-1P: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

1Q.) *Chrysomya* sp.

1R.) *Acytolepis* sp.

1S.) *Eurema* sp.

Plate 1Q-1S: Pollinator diversity recorded during the roving survey (2018-2019) (100mm Macrolens; Magnification: 1X)

districts, whereas Margalef's index was high in Thrissur district (5.30) followed by Palakkad district (5.01) and Ernakulam (3.28) districts. Though the species richness is high in the Thrissur district (33), Menhinick's index was found low as compared to the Palakkad district since the effect of sample size becomes reduced in Menhinick's index as compared to the Margalef's index, in which effect of sample size remains the same.

Pielou's evenness index (J) was found to be high in the Thrissur district (0.74) followed by the Ernakulam (0.73) and Palakkad districts, which showed that pollinator species were more uniformly distributed in the Thrissur district.

Table 8. Diversity indices of all pollinators in three districts of central Kerala

Serial Number	Diversity Indices	Thrissur	Palakkad	Ernakulam
1	Taxa (S)	33	29	17
2	Individuals	416	267	131
3	Dominance (D)	0.09	0.14	0.17
4	Simpson (1-D)	0.90	0.85	0.82
5	Shannon (*H'*)	2.59	2.29	2.07
6	Brillouin (H_B)	2.47	2.14	1.89
7	Menhinick	1.61	1.77	1.48
8	Margalef	5.30	5.01	3.28
9	Evenness (*J*)	0.74	0.68	0.73
11	Berger-Parker	0.13	0.23	0.26

4.1.3. The relative abundance of solitary pollen bees in three districts of central Kerala

A total of 23 solitary pollen bee species were recorded during the roving survey from three districts *viz*., Thrissur, Palakkad, and Ernakulam. The relative species abundance of solitary pollen bees was calculated to assess the species level abundance in three districts surveyed from March 2018 to December 2019 (Table 9).

The small carpenter bee, *C. hieroglyphica* (25.94 %) was more abundant in the Thrissur district which was followed by *C. smaragdula* (19.81 %), *N. curvipes* (18.39

%), *B. picitarsis* (16.98 %), *Halictus* sp. (7.07 %), *M. disjuncta* (3.77 %), *L. serenum* (2.35 %), *A. zonata* (2.35 %), *B. mixta* (1.41 %), *X. ruficornis* (0.47 %), *X. fenestrata* (0.47 %), *Thyreus* sp. 1 (0.47 %) and *Thyreus* sp. 2 (0.47 %). Among these solitary pollen bees, *X. ruficornis, X. fenestrata, Thyreus* sp. 1 and *Thyreus* sp. 2 were represented as singleton species in the selected cucurbitaceous ecosystem as they were very rare and caught only single time during the sweep net collection from Thrissur district. *B. mixta* was represented as tripleton species that was caught three times in the sweep net collection.

The small carpenter bee, *C. hieroglyphica* (32.96 %) was found more relatively abundant in the Ernakulam district which was followed by *C. smaragdula* (27.47 %), *B. picitarsis* (12.08 %), *Halictus* sp. (3.29 %), and *N. curvipes* (3.29 %). Palakkad district was high in species richness as compared to the Thrissur district as it was represented by 19 species of solitary bees in the sweep net collection. Most of the solitary bee species other than the small carpenter bees (*C. hieroglyphica* & *C. smaragdula*) and allodapine bee (*B. picitarsis*) were grouped as singleton, doubleton, or tripleton species. Singleton species *viz.*, *L. interstitialis, Nomiapis* sp. and *Lipotriches* sp. were only documented from the Palakkad district in the present study. Doubleton species *viz*., *Ceratina* sp., *H. elliotti*, and *G. thoracica* were also documented from the Palakkad district only.

The small carpenter bee, *C. smaragdula* (40.62 %) was more relatively abundant in the Ernakulam district which was followed by *C. hieroglyphica* (28.12 %), *C. binghami* (9.37 %) and *B. picitarsis* (6.25 %). The species richness of solitary pollen bees was low in the Ernakulam district (8) as compared to that of the Thrissur (13) and Palakkad (19) districts. Solitary bee species *viz*., *A. zonata*, *Coelioxys* sp. and *Megachile* sp.1 were grouped into singleton species in the sweep net collection of Ernakulam district.

Table 9. The relative species abundance of solitary pollen bees collected from three different districts

Species	Districts					
	Thrissur		Palakkad		Ernakulam	
	No.	%	No.	%	No.	%
Braunsapis picitarsis	36	**16.98**	11	**12.08**	2	6.25
Braunsapis mixta	3	1.41	2	2.19	0	0.00
Ceratina smaragdula	42	**19.81**	25	**27.47**	13	**40.62**
Ceratina binghami	0	0.00	1	1.09	3	9.37
Ceratina hieroglyphica	55	**25.94**	30	**32.96**	9	**28.12**
Ceratina sp.	0	0.00	2	2.19	0	0.00
Xylocopa ruficornis	1	0.47	1	1.09	0	0.00
Xylocopa fenestrate	1	0.47	1	1.09	0	0.00
Amegilla zonata	5	2.35	1	1.09	1	3.12
Thyreus sp. *1*	1	0.47	1	1.09	0	0.00
Thyreus sp. *2*	1	0.47	0	0.00	0	0.00
Halictus sp.	15	7.07	3	3.29	0	0.00
Lasioglossum serenum	5	2.35	2	2.19	0	0.00
Nomia curvipes	39	**18.39**	3	3.29	0	0.00
Hoplonomia elliotti	0	0.00	2	2.19	0	0.00
Gnathonomia thoracica	0	0.00	2	2.19	0	0.00
Leuconomia interstitialis	0	0.00	1	1.09	0	0.00
Nomiapis sp.	0	0.00	1	1.09	0	0.00
Lipotriches sp.	0	0.00	1	1.09	0	0.00
Coelioxys sp.	0	0.00	0	0.00	1	3.12
Megachile disjuncta	8	3.77	0	0.00	0	0.00
Megachile sp. *1*	0	0.00	1	1.09	1	3.12
Megachile sp. *2*	0	0.00	0	0.00	2	6.25
Total	**212**	**-**	**91**	**-**	**32**	**-**
Species Number	**13**	**-**	**19**	**-**	**8**	**-**

4.1.4. Comparison of diversity indices of solitary pollen bees among three districts of Central Kerala

Diversity indices were used to study the diversity, richness, and evenness of the solitary pollen bees collected from three districts of Central Kerala (Table 10). The diversity of solitary pollen bees collected was measured by Simpson's diversity index (1-D), Shannon-Weiner index (*H'*), Brillouin index (H_B), and Berger-Parker index. Whereas, the species richness of each district was measured by Menhinick and Margalef's indices. The evenness of the species distributed in the surveyed area was calculated by Pielou's evenness (*J*) index.

The diversity study revealed that the Palakkad district has a high number of taxa (19) followed by Thrissur (13) and Ernakulam (8) districts. The total number of individuals in the sweep net collection was high in Thrissur district (212) as compared to Palakkad (91) and Ernakulam (32) districts. Simpson's Dominance (D) was found to be high in Ernakulam district (0.26) followed by Palakkad (0.20) and Thrissur (0.17), which clearly showed that the diversity of species is low in Ernakulam district as compared to that of the other two districts. This was confirmed with the Simpson's diversity index (1-D) in which Thrissur district showed the highest value (0.82) followed by Palakkad (0.79) and Ernakulam (0.73) districts. The Simpson's diversity index (1-D) values showed that the Thrissur and Palakkad districts were more species-rich and uniformly distributed when compared to the Ernakulam district. Shannon diversity index (*H'*) for all three districts ranged from 1.50 to 2.10 which revealed that the Ernakulam district was very low in species diversity (1.61) followed by the Thrissur (1.92) district. Whereas Palakkad district was found to be more diverse (2.06) in species distribution when compared to the other two districts. Brillouin index (H_B) was found the highest in the Thrissur district (1.83) and the lowest in the Ernakulam district (1.34), which confirmed that the species abundance was very high in the Thrissur district. Though the Brillouin index of the Thrissur (1.83) and Palakkad (1.81) districts were almost similar, species abundance was higher in the Thrissur district related to the sample size. The Berger-Parker index was found higher in the Ernakulam district (0.40) followed by Palakkad (0.32) and Thrissur (0.25) districts, which showed that in the Ernakulam district the pollinator community is dominated by the most common species.

The species richness of the three districts was compared with the help of Menhinick's index and Margalef's index. In the present study, Menhinick's index was high in the Palakkad district (1.99) followed by Ernakulam (1.41) and Thrissur (0.89) districts. Margalef's index was high in Palakkad district (3.99) followed by Thrissur district (2.24) and Ernakulam (2.04) districts. Both indices showed that the Thrissur district was highly species-rich as compared to the other two districts.

Pielou's evenness index (J) was found to be high in the Ernakulam district (0.77) followed by the Thrissur (0.75) and Palakkad (0.70) districts, which showed that pollinator species were more uniformly distributed in the Ernakulam district. Though the Palakkad district is highly species-rich, evenness (J) is low due to more number of singleton and doubleton species caught in sweep net collection. Although Species were more uniformly distributed and abundant in the other two districts, species richness is low. The overall diversity indices proved that the Palakad district is rich in solitary pollen bee species.

Table 10. Diversity indices of solitary bee pollinators collected from central Kerala

Sl. No.	Diversity Indices	Thrissur	Palakkad	Ernakulam
1	Taxa (S)	13	19	8
2	Individuals	212	91	32
3	Dominance (D)	0.18	0.20	0.26
4	Simpson (1-D)	0.82	0.79	0.74
5	Shannon (*H'*)	1.93	2.07	1.62
6	Brillouin (H_B)	1.83	1.82	1.35
7	Menhinick	0.89	1.99	1.41
8	Margalef	2.24	3.99	2.02
9	Evenness (*J*)	0.75	0.70	0.78
11	Berger-Parker	0.26	0.33	0.41

4.1.5. Molecular characterization of solitary pollen bees

A total of 23 solitary pollen bees were recorded during the roving survey conducted in the selected cucurbitaceous ecosystem. Morphological characterization of all solitary bees was done with the help of taxonomists, Dr. Amala Udayakumar (Scientist, NBAIR), Dr. Jobiraj T. (Assistant Professor, Govt. College Kodenchery, Kozhikode), and Dr. P. Girish Kumar (Scientist, Zoological Survey of India, Kozhikode). Molecular characterization of different species of solitary pollen bees coming under the same genera was done to confirm their identity.

4.1.5.1. Isolation of genomic DNA

The genomic DNA of 15 solitary bee species was isolated using DNeasy blood and tissue kit method. The quantity and quality of the isolated DNA were analysed and absorbance values were recorded at $A_{260/280.}$ Absorbance values ranged between 1.80 to 2.03, and the quantity of DNA ranged from 70.13 µg/ml to 121.87 µg/ml (Table 11).

4.1.5.2. Amplification of barcode locus at mtCO1

The universal barcode region or the mtCO1 region of the specimen was amplified using the universal barcode primers specified for hymenopteran insects. The length of the amplified fragment was between 600 to 750 bp for the solitary bees. The PCR products were assessed for amplification by 1.2 per cent agarose gel electrophoresis. Bands were formed in the region between 700-800 bp for indicating amplification.

4.1.5.3. Sequencing of PCR products

The sequencing of the PCR products, which formed a single good band was outsourced to Biokart India Pvt. Ltd., Bengaluru, and Agrigenome Labs Pvt. Ltd., Cochin. Details of DNA sequences obtained are presented below (Table 12).

Table 11. Quantity and quality assessment of isolated DNA

Sl. No.	Solitary pollen bee species	Quality ($A_{260/280}$)	Quantity (µg/ ml)
1	*Braunsapis picitarsis*	1.85	87.34
2	*Braunsapis mixta*	1.90	78.50
3	*Ceratina binghami*	1.93	70.13
4	*Ceratina hieroglyphica*	2.03	90.17
5	*Ceratina smaragdula*	1.83	91.23
6	*Halictus* sp.	1.81	121.87
7	*Lasioglossum serenum*	2.00	75.08
8	*Nomia curvipes*	2.01	82.15
9	*Xylocopa fenestrata*	1.89	99.20
10	*Xylocopa ruficornis*	1.85	95.16
11	*Megachile disjuncta*	1.80	72.56
12	*Megachile* sp. *1*	1.93	82.07
13	*Megachile* sp. *2*	2.00	101.53
14	*Thyreus* sp. 1	1.83	90.05
15	*Thyreus* sp. 2	1.80	72.55

Table 12. DNA sequence data of solitary pollen bees

Braunsapis picitarsis
ATAAAGATATTGGTATACTATATATTATATTTGCTTTATGATCTGGAATAA
TTGGATCTTCTATAAGATTAATTATTCGAATAGAACTAGGAATTCCGGGAA
GATGAATTAACAATGATCAGATTTATAATTCTATAGTAACTTCTCATGCAT
TTTTAATAATTTTTTTTATAGTAATACCATTTATAATTGGAGGATTTGGTAA
TTGATTAATTCCCTTAATATTAGGATCCCCTGACATAGCTTTTCCTCGAAT
AAATAACATTAGATTTTGATTACTTCCTCCTTCATTATTATTATTATTATTA
AGTAATTTATTTAACCCTAGGCCTGGAACAGGTTGGACTGTATATCCTCCT
TTATCATCATATATATTTCATTCATCTCCATCAGTTGATTTAACAATTTTTT
CTTTACATATATCAGGAATTTCATCAATTTTAGGAGCAATAAATTTTATAG
TTACCATTATAATAATAAAAAACTTATCTTTAAATTACGATTATATTACTT
TATTTTCTTGATCAGTTTTTATTACTGCAATTTTATTATTATTATCATTACC
AGTATTAGCAGGAGCAATTACCATACTATTATTTGATCGTAATTTTAATAC
ATCTTTTTTTGATCCT
Braunsapis mixta
ATAAAGATATTGGTATATTATATATTATATTTGCTTTATGATCTGGTATGA
TTGGATCTTCAATAAGATTAATTATTCGAATGGAATTAGGAATTCCAGGA
AGTTGAATTAATAATGATCAAATTTATAATTCAATAGTGACTTCTCATGCA
TTTTTAATAATTTTTTTTATGGTTATACCTTTTATAATTGGGGGATTTGGTA
ATTGATTAATTCCTTTAATATTAGGATCTCCTGATATAGCTTTTCCACGAAT
AAATAATATTAGATTCTGATTACTTCCTCCTTCATTATTATTATTATTATTA
AGAAATTTATTTAATCCAAGTCCTGGTACAGGATGGACTGTTTATCCTCCT
TTATCTTCTTATATATTTCATTCATCTCCATCAGTTGATTTAACAATTTTTTC
ATTACATATATCAGGAATTTCATCAATTTTAGGTGCTATAAATTTTATAGT
AACAATTATAATAATAAAAAATTTATCTTTAAATTATGATTATATTACTTT
ATTTTCTTGATCAGTTTTTATTACAGCAATTTTATTATTATTATCATTACCA
GTTTTAGCTGGTGCAATTACTATATTGTTATTTGATCGGAATTTTAATACAT
CTTTTTTTGATCCTATAGG

Ceratina binghami
TATAAGATTAATTATTCGAATAGAATTAAGAATTCCTGGAAATTGAATTA ATAATGATCAAATTTATAATTCTTTAGTTACAGCTCATGCTTTTTTAATGAT TTTTTTTATAGTAATACCTTTAATAATTGGAGGATTTGGTAATTGATTAATT CCATTAATATTAGGTTCTCCAGATATATCTTTTCCTCGATTAAATAATATTA GATTTTGATTATTACCACCTTCTTTATTATTATTATTATCAAGAAATTTATT TACTTTAAGTCCAGGAACTGGTTGAACTGTATATCCACCATTATCATTATA CTTATATCATTCATCTCCTTCAGTTGATTTAACTATTTTTTCTTTACATATAT CTGGTATTTCATCAATTTTAGGTGCTATTAATTTTATAGTAACTATTATAAT AATAAAAAATATTTCTATTAATTATGATAATATTAGATTATTTTCTTGATC AGTATTTATTACAGCTATTTTATTATTATTATCTTTACCTGTATTAGCAGGT GCTATTACTATATTACTATTTGATCGTAATTTAAATACATCATTTTTTGATC CAATAGGAGGAGGAGATCCTGTTTTATATCAACATTTATTTTGATTTTTTG G
Ceratina hieroglyphica
TAAAGATATTGGAATTTTATATATTATATTTGCTATATGATCAGGAATAAT TGGAGCATCAATAAGTTTAATTATTCGAATAGAATTAAGAACACCTGGTA ATTGAATTAGAAATGATCAAATTTACAATTCCCTTGTTACTGCTCACGCTT TCCTAATAATTTTTTTTATAGTTATACCATTCATAATTGGTGGATTTGGAAA TTGATTAATTCCTTTAATATTAGGTTCACCTGATATATCATTTCCACGATTA AATAATATTAGATTTTGATTATTACCACCTTCATTATTATTATTATTATCAA GAAATTTATTTTCTATAAGTCCAGGAACTGGATGAACAGTTTATCCTCCTT TATCATCTTATTTATTTCATTCTTCACCATCTGTAGATTTAGCTATTTTTTCA TTACATATATCAGGAATTTCATCAATTTTAGGAGCCATTAATTTTATAGTT ACAATTATATTAATAAAAAATATCTCTTTAAATTATGATAATATCCCATTA TTTTCTTGATCAATTTTTATCACTGCAATTTTATTATTACTTTCATTACCAG TATTAGCAGGAGCTATTACTATATTATTATTTGATCGTAACTTAAATACCT CTTTTTTTGATCCTATAGGAGGAGGTGATCCAATTTTATATCAACATTTATT TTGATTTTTTG

Ceratina smaragdula
AGGAATAATTGGTGCATCTATAAGATTAATTATTCGAATAGAATTAAGAA
TTCCTGGAAATTGAATTAATAATGATCAAATTTATAATTCTTTAATTACAG
CTCATGCTTTTTTAATAATTTTTTTTATAGTAATACCTTTTATAATTGGAGG
ATTTGGTAATTGATTAATTCCATTAATATTAGGTTCTCCAGATATATCCTTT
CCTCGATTAAATAATATTAGATTTTGATTATTACCTCCTTCTTTATTATTAT
TATTATCAAGAAATTTATTTACTTTAAGTCCAGGAACTGGTTGAACTGTAT
ATCCACCATTATCATTATATTTATATCATTCATCTCCTTCAGTTGATTTAAC
TATTTTTTCTTTACATATATCTGGTATTTCTTCAATTTTAGGTGCTATTAATT
TTATAGTAACTATTATAATAATAAAAAACATTTCTTTAAATTATGACAATA
TCAGATTATTTTCTTGATCAGTATTTATTACAGCTATTTTATTATTATTATC
TTTACCTGTATTAGCAGGTGCTATTACTATATTATTATTTATCGTAATTTAA
ATACATCATTTTTTGATCCAATAGGAGGAGGAGATCCTGTTTTATATCAAC
ATTTATTTTGATTTTTTGGT
***Halictus* sp.**
ATAAAGATATTGGAATACTTTATTTCATTTTTGCTATATGATCAGGAATAA
TTGGTGCTTCATTAAGAATAATTATTCGTATAGAATTAAGAACTCCAGGTA
GATGAATTAATAATGATCAAATTTATAATACTATTGTTACTTCCCATGCTT
TTGTAATAATTTTTTTTATAGTTATACCATTTATAATTGGAGGTTTTGGAAA
CTGACTTGTACCTTTAATAATTGGAGCTCCTGATATAGCTTTCCCACGTAT
AAATAATATAAGATTTTGATTATTAATTCCTTCTCTATTTATACTTTTAATA
AGAAGAATTTTATCAACAGGATCAGGAACAGGATGAACTATTTACCCTCC
CTTATCTTCAATTATATATCACTCATCCTCTTCAGTTGATTTTACTATTTTTT
CTCTTCATATTGCTGGAATTTCTTCAATTATAGGAGCTATTAATTTCATTGT
TTCAGTTCTTTTAATAAAAAATGTTTCTCTTAAATTAAATCAAATTCCTTTA
TTTCCATGATCAGTAAAAATTACTGCTATTCTATTACTTTTATCATTACCTG
TATTAGCAGGTGCTATTACTATATTATTAACTGACCGAAATTTAAATACAT
CTTTTTTTGACCCTTCAGGAGGAGGAGACCC
Lasioglossum serenum
GAATGGAATAATTGGAGCATCCCTAAGTATAATTATTCGAATAGAATTAA
GAGTCCCCGGAAAATGAATTAATAATGATCAAGTATTTAACACCATCGTC
ACATCCCACGCTTTCATTATAATTTTTTTCATGGTTATACCTTTTATAATTG
GAGGATTTGGAAACTGATTAGTCCCTCTTATAATTGGAGCCCCTGATATAG

CCTTTCCTCGTATAAATAACATAAGATTTTGATTATTAACTCCTTCACTATT
ACTCTTAATCTTTAGTTCTATATCTACAGGAACAGGTACGGGATGAACAAT
TTACCCTCCATTATCATCTATTACCTACCATTCTTCTAACTCTGTCGATTTT
ACTATCTTTTCTCTTCATATTGGAGGAATATCCTCCATTATAGGAGCAATT
AACTTCATTGTATCAATTATAATAATAAAAAATATTTCAATCAATATAGAT
AAAATCCCTTTATTTCCTTGATCAGTAAATATTACAGCTATTTTATTAGTA
GTATCCCTCCCAGTTTTAGCGGGGGCTATTACCATACTACTAGCAGATCGA
AACTTAAATACTTCATTTTTTGACCCCTCAGGAGGAGGAGACCCTATTTTA
TACCAACATTTATTCTGATTTTTTGG

Nomia curvipes

CATAAAGATATTGGAATATTATATTTCATCCTTGCAATATGATCAGGAATA
TTAGGATCTTCATTAAGAATAATTATTCGAATAGAATTAAGAATTCCAGGT
TCATGAATTAATAATGATCAACTTTATAATACAATTATTACAGCTCATGCA
TTTTTAATAATTTTTTTTATAGTTATACCATTTATAATTGGTGGATTTGGAA
ATTGATTAATTCCATTAATAATAGGAACACCAGATATAGCTTTTCCACGAA
TAAATAATTTAAGGTTTTGATTAATAGTTCCATCATTATTTTTATTAATTAT
TAGAACTATTTCAGGATCAGGTATAGGAACAGGATGAACTGTATATCCTC
CTTTATCATCTATTTTATTTCATTCCTCAATATCAGTTGATTATGGAATTAT
TTCTCTTCATATTGCAGGAATATCATCAATTCTAGGAGCAATAAATTTTAT
TACAACAATTTATTATTCAAAAAATATTTCTATAAATTATAATCAAATTTC
ACTTTTTCCATGATCAGTAATTATTACTGCAATTTTATTATTATTATCATTA
CCAGTTCTTGCAGGAGCAATTACTATATTATTAACAGATCGAAATTTAAAT
ACTTCATTTTTTGAACCATCTGGAGGTGGTGATCCAATTCTATATCAACAT
TTATTTTGATTTTTTGGTCACCCTGAAAGTT

Xylocopa fenestrata
ATAAAGATATTGGTATATTATATATTATTTTAGCTTTATGAGCAGGTATAT
TAGGAACATCAATAAGAATAATTATTCGTATAGAATTAAGAATTCCTGGA
TCCTGAATTAATAATGATCAAATTTATAATTCAATAATTACAGCTCATGCA
TTTTTAATAATTTTTTTTATAGTAATACCTTTTATAATTGGTGGATTTGGAA
ATTGATTAATTCCAATAATATTAGGCTTACCTGATATAGCTTTTCCACGAA
TAAATAATATTAGATTTTGATTATTACCACCTTCACTTATTTTATTAATTTT
AAGAAATTTATTTAATCCAAGACCTGGAACTGGTTGAACTATTTATCCTCC
TTTATCATCATTTTTATATCATTCATCTCCTGCTGTAGATTTAATAATTTTTT
CTTTACATATTTCTGGAATTTCATCAATTATAGGAGCTATAAATTTTATTGT
GACAATTATAATAATAAAAAATATTTCAATAAATTATGATAAAATTAATTT
ATTTGCATGATCAGTATTTATTACAGCTATTTTATTATTATTATCATTACCT
GTTTTAGCTGGAGCAATTACTATATTATTATTTGATCGAAATTTTAATACA
TCATTTTTTGATCCAATAG
Xylocopa ruficornis
ATAAAGATATTGGTATATTATATATTATTTTAGCTTTATGAGCAGGTATAT
TAGGAACATCAATAAGAATAATTATTCGTATAGAATTAAGAATTCCTGGA
TCCTGAATTAATAATGATCAAATTTATAATTCAATAATTACAGCTCATGCA
TTTTTAATAATTTTTTTTATAGTAATACCTTTTATAATTGGTGGATTTGGAA
ATTGATTAATTCCAATAATATTAGGCTTACCTGATATAGCTTTTCCACGAA
TAAATAATATTAGATTTTGATTATTACCACCTTCACTTATTTTATTAATTTT
AAGAAATTTATTTAATCCAAGACCTGGAACTGGTTGAACTATTTATCCTCC
TTTATCATCATTTTTATATCATTCATCTCCTGCTGTAGATTTAATAATTTTTT
CTTTACATATTTCTGGAATTTCATCAATTATAGGAGCTATAAATTTTATTGT
GACAATTATAATAATAAAAAATATTTCAATAAATTATGATAAAATTAATTT
ATTTGCATGATCAGTATTTATTACAGCTATTTTATTATTATTATCATTACCT
GTTTTAGCTGGAGCAATTACTATATTATTATTTGATCGAAATTTTAATACA
TCATTTTTTGATCCAATAGGTGGTGGAGATCCAATTTTATTTCAACATTTAT
TTTGATTTTTTGGTCAC

Megachile disjuncta
TAAAGATATTGGTATTATATATATAATTTTTGCTTTATGATCTGGAATAAT
TGGTTCTTCTTTAAGAATAATTATTCGTATAGAATTAAGAATTCCTGGTTC
ATGAATTAAAAATGATCAAATTTATAATTCAATTGTTACTGCTCATGCTTT
TTTAATAATTTTTTTTTTAGTTATACCTTTTATAATTGGAGGATTTGGAAAT
TGATTAATACCTTTAATAATTGGAGCTCCTGATATAGCCTTTCCTCGAATA
AATAATATTAGATTTTGATTACTTCCTCCTTCTCTTATTTTATTATTAATTA
GAAATTTATTAAATCCTAGACCTGGAACAGGATGAACAATTTATCCTCCCT
TATCTTTATATCTATATCATCCATCTCCATCAGTTGATTTAACTATTTTTTC
TCTTCATATATCTGGTGTATCATCAATTATTGGATCCTTAAATTTTATTGTA
ACTATTCTAATAATAAAAAATTTTTCTTTAAATATTAGAAAAATACCTTTA
TTTCCTTGATCAATTTTAATTACTACTATTCTTCTTTTATTATCATTACCTGT
CTTAGCTGGAGCTATTACTATACTTCTTTTTGATCGAAATTTAAATACTTCA
TTTTTTGATCCCATAGGAGGAGGAGATCCGATTTTATATCAACATTTATTT
TGATTTTTTGGTCA
***Megachile* sp. 1**
TATGGTCAGGAATAATTGGATCTAGTATATCAATAATTATTCGAATAGAAT
TAAGTACACCAGGATCATGAATTAAAAACGACCAAATTTACAATTCTATT
GTAACAGCACACGCATTTCTAATAATTTTTTTTTTAGTTATGCCATTTATAA
TTGGTGGTTTTGGTAATTGGTTAATACCATTAATAATTGGAGCTCCGGATA
TAGCCTTCCCTCGAATAAATAATGTAAGATTTTGATTATTGCCCCCATCAT
TAATCTTACTATTAATAAGAAATTTATTAACTCCTAGACCAGGGACAGGAT
GAACTGTATACCCTCCATTATCTTTATATATATTTCACCCTTCACCATCAGT
AGATCTAACAATTTTTTCATTACACTTATCAGGAATTTCATCAATCATTGG
TTCTTTAAATTTTATGGTAACAATTTTAATAATAAAAAATAATTCATTAAA
TTATAGACAAATAACATTATTCCCTTGATCTGTTTTTATTACAACAGTATT
ATTATTATTATCATTACCAGTATTAGCAGGAGCAATCACAATACTATTATT
TGATCGAAATTTAAATACCTCATTTTTTGATCCTATGGGAGGAGGAGATCC
AATTTTATATCAACATTTATTTTGATTTTTTGGT

***Megachile* sp. 2**

ATAAAGATATTGGCATACTCTACATAATCTTTGCACTATGGTCAGGAATAA
TTGGATCTAGTATATCAATAATTATTCGAATAGAATTAAGTACACCAGGAT
CATGAATTAAAAACGACCAAATTTACAATTCTATTGTAACAGCACACGCA
TTTCTAATAATTTTTTTTTTAGTTATGCCATTTATAATTGGTGGTTTTGGTA
ATTGGTTAATACCATTAATAATTGGAGCTCCGGATATAGCCTTCCCTCGAA
TAAATAATGTAAGATTTTGATTATTGCCCCCATCATTAATCTTACTATTAA
TAAGAAATTTATTAACTCCTAGACCAGGGACAGGATGAACTGTATACCCT
CCATTATCTTTATATATATTTCACCCTTCACCATCAGTAGATCTAACAATTT
TTTCATTACACTTATCAGGAATTTCATCAATCATTGGTTCTTTAAATTTTAT
GGTAACAATTTTAATAATAAAAAATAATTC
ATTAAATTATAGACAAATAACATTATTCCCTTGATCTGTTTTTATTACAAC
AGTATTATTATTATTATCATTACCAGTATTAGCAGGAGCAATCACAATACT
ATTATTTGATCGAAATTTAAATACCTCATTTTTTGATCCTATGGGAGGAGG
AGATCCAATTTTATATCAACATTTATTTTGATTTTTT

***Thyreus* sp. 1**

ATAAAGATATTGGAATTTTATATATAATATTTGCTATATGATCAGGAATTA
TAGGGACAGCAATGAGATTTTTAATTCGATTAGAACTTAGAATTCCAGGG
AAATGAATTAATAATGACCAGTTATATAACTCTATTGTTACTTCTCATGCT
TTTATTATAATTTTTTTTTTAGTAATACCTTTTTTAATTGGAGGATTTGGAA
ATTGATTAATCCCAATAATACTTGGATCTCCAGATATAGCTTTTCCACGAA
TAAATAATATTAGATTTTGATTACTACCTCCCTCATTAATATTATTAATACT
AAGAAATACTTTTAAAATAACAATAGGGACTGGGTGAACTGTTTACCCCC
CTTTATCATCATTAATATATCATAATAGACCTTCGGTTGATCTAAGAATTT
TTTCATTACATATATCTGGTGTCTCTTCTATTTTAGGGGCAATAAATTTTAT
AGTAACAATTATATTAATAAAGAATTTTAGATTAAATTATGATCAATTAAA
TTTATTTTCTTGATCTGTTTTTATTACAGCAATTTTATTATTAGTATCACTA
CCAGTTCTTGCAGGTGCAATTACAATATTATTATTTGATCGAAATTTAAAT
ACAAGGTTTTTTGACCCAATAGGAGGGGGAGACCCAATTTTATATCAACA
TTTGTTTTGATTTTTTGGTC

***Thyreus* sp. 2**

TAAAGATATTGGAGTACTTTATATATTGTTCGCTTTATGATCAGGAATAAT
TGGAACATCAATAAGATTTTTAATTCGATTAGAATTAAGAATACCAGGAA
AATGAATTAGAAACGATCAATTATATAATTCTATTGTAACTGCACATGCTT
TTATTATAATTTTTTTTTTAGTTATACCGTTTTTAATTGGTGGATTTGGTAA
TTGATTAGTTCCAATAATGTTAGGATCTCCTGATATAGCTTTTCCTCGAAT
AAATAATGTAAGTTTTTGATTATTACCACCATCATTAATTTTATTATTAAC
AAGAAATTTTTTTAAAACTACAATAGGAACTGGATGAACATTATATCCTCC
ACTATCATCATCATTATATCATAATAGACCTTCAGTTGATATTGGAATTTT
TTCATTACATATATCAGGAATTTCTTCAATTTTAGGTGCAATAAATTTTAT
GGTAACAATTATATTAATAAAAAATTTTAGTTTAAATTATGATCAGTTAAA
TTTATTTTCTTGATCAGTTTATATTACAGCAATTTTATTATTATTTTCATTAC
CTGTATTGGCTGGAGCAATTACTATATTATTATTTGATCGAAATTTCAATA
CAAGATTTTTTGATCCAATAGGTGGTGGAGATCCGATTTTATATCAACATT
TATTTTGATTTTTTGGTCACCT

4.1.5.3. Sequence homology analysis of solitary pollen bees

The trimmed forward and reverse sequences were combined using the CAP3 sequence assembler to develop the contigs. The homology of the sequences was analysed using the Basic Local Alignment Search Tool for Nucleotide (BLASTn) of the NCBI database. The sequences which showed maximum query cover, per cent identity, and zero E value were compared from the database and species identity was confirmed. Details of the sequence homology of solitary pollen bees are presented below (Table 13).

Table 13. Homology of the sequences generated from the NCBI database

Sample code	Species	Query coverage	Per cent identity	E value	Corresponding hits	Corresponding species
BRPIC	*B. picitarsis*	97.00	100.00	0.0	MW135303.1	*B. picitarsis*
BRMX	*B. mixta*	97.00	96.00	0.0	MZ619049.1	*B. mixta*
CRBI	*C. binghami*	98.00	99.84	0.0	KT960843.1	*C. binghami*
SPB2	*C. hieroglyphica*	96.00	100.00	0.0	JF866184.1	*Ceratina* sp.
CSMA	*C. smaragdula*	100.00	98.59	0.0	NC064404.1	*C. smaragdula*
HALSP	*Halictus* sp.	94.00	99.51	0.0	KY834543.1	*Halictus* sp.
LASER	*L. serenum*	98.00	99.20	0.0	JF866341.1	Hymenoptera species
NCURV	*N. curvipes*	83.00	100.00	0.0	KY072357.1	Apoidea species
XFEN	*X. fenestrata*	97.00	91.00	0.0	KM585613.1	*X. virginica*
XRUFI	*X. ruficornis*	91.00	98.41	0.0	MK904708.1	*X. nasalis*
MDIS	*M. disjuncta*	99.00	98.24	0.0	ON331717.1	*M. disjuncta*
MLER	*Megachile* sp. 1	94.00	99.00	0.0	MN856202.1	*Megachile* sp.
LBRM	*Megachile* sp. 2	99.00	99.01	0.0	MN856202.1	*Megachile* sp.
THYR	*Thyreus* sp. 1	91.00	98.56	0.0	MK904753.1	*Thyreus* sp.
THSP	*Thyreus* sp. 2	96.00	96.71	0.0	MK904768.1	*Thyreus* sp.

4.1.5.4. Generation of accession number and barcodes for the solitary pollen bee species

The 15 DNA sequences obtained were combined and processed using BioEdit sequence alignment editor and MEGA7 software, and uploaded at NCBI GenBank to generate the accession numbers. The sequences were then uploaded to BOLD systems and illustrative DNA barcodes were developed for 15 species. A BOLD BIN number was obtained from BOLD systems for each barcode. Details on the accession number and BOLD BIN number are presented below (Table 14).

Table 14. Details of accession number and BOLD Bin number generated for solitary pollen bees

Sl. No.	Species	Accession Number	BIN Number
1	*Braunsapis picitarsis*	MW856777	BOLD: AET3422
2	*Braunsapis mixta*	MW856776	BOLD: AEU4376
3	*Ceratina binghami*	MW856668	BOLD: AAF1368
4	*Ceratina hieroglyphica*	MW028134	BOLD: ABZ0918
5	*Ceratina smaragdula*	MW856669	BOLD: AAF1368
6	*Halictus* sp.	MW868391	BOLD: AAX2275
7	*Lasioglossum serenum*	MW872014	BOLD: AAN4355
8	*Nomia curvipes*	OK287373	BOLD: AAI9941
9	*Xylocopa fenestrata*	OM149840	BOLD: AET4957
10	*Xylocopa ruficornis*	OK272467	BOLD: AET4957
11	*Megachile disjuncta*	OK287393	BOLD: AAJ3088
12	*Megachile* sp. 1	OK287391	BOLD: AAK7030
13	*Megachile* sp. 2	OK287392	BOLD: AAK7030
14	*Thyreus* sp. 1	OK287376	BOLD: ACV4821
15	*Thyreus* sp. 2	OK287374	BOLD: ACA6412

4.2. Determination of the peak foraging time of solitary pollen bees

The foraging activity of bee pollinators was observed to study the temporal variations in their foraging behavior in three different seasons *viz*., post-monsoon (2018), summer (2019) and monsoon (2019) . The cucurbit crops such as bitter gourd (var. Preethi) and oriental pickling melon (var. Saubhagya) were raised separately to record the mean number of solitary pollen bees per square meter area (N=100 days), the total abundance of pollinators in 100 per cent flowering stage of crops (N=5 days), and the number of visit by pollinators in crops at 25, 50, 75 and >90 per cent flowering stage (N=5 days).

4.2.1 Mean number of solitary pollen bees per square meter area in bitter gourd ad oriental pickling melon ecosystems at three different seasons

The data revealed that the mean number of solitary pollen bees per square meter area (N=100 days) in bitter gourd ecosystem was the highest during 9.00 AM to 10.00 AM with an average of 446±40.90 solitary bee pollinators in the post-monsoon (2018) (Table 15). Whereas, the least mean number of solitary pollen bees (206.40±29.70) was recorded during 6.00 AM to 7.00 AM. In summer (2019), the highest mean number of solitary pollen bees was recorded during 9.00 AM to 10.00 AM with an average of 467.20±33.50, whereas the least mean number of solitary pollen bees was recorded during 6.00 AM to 7.00 AM with an average of 205.80±27.50. In monsoon (2019), the highest mean number of solitary pollen bees was recorded during 9.00 AM to 10.00 AM with an average of 353±22.60, whereas the least mean number of solitary pollen bees were recorded during 6.00 AM to 7.00 AM with an average of 124.20±12.90. Thus, 9.00 AM to 10.00 AM was found to be the peak hour of foraging for the solitary pollen bees in the bitter gourd ecosystem during post-monsoon (2018), summer (2019) and monsoon (2019) seasons. The bitter gourd flowers were recorded with the least mean number of solitary bees during the early hours (6.00 AM to 7.00 AM) in all three seasons.

The mean number of solitary pollen bees per square meter area in the oriental pickling melon ecosystem was observed for post-monsoon (2018), summer (2019) and monsoon (2019) seasons. In post-monsoon (2018), the highest mean number of solitary pollen bees were recorded during 9.00 AM to 10.00 AM with an average number of 369.40±16.40 solitary bees per square meter area, whereas the least mean number of solitary pollen bees were recorded during 11.00 AM to 12.00 PM with an average of 177.6±14.1 solitary bees per square meter. In summer (2019), the highest mean number of solitary pollen bees per square meter area was found to be 386.60±20.2 during 10.00 AM to 11.00 AM, whereas the least mean number of solitary pollen bees was found to be 168.40±14.30 during 11.00 AM to 12.00 PM. In monsoon (2019), the highest mean number of solitary pollen bees was recorded during 9.00 AM to 10.00 AM with an average of 343.80±27.60, whereas the least mean number of solitary pollen bees was recorded during 11.00 AM to 12.00 PM with an average of 186.8±13.6 solitary bees (Table 16).

Table 15. Mean number solitary pollen bees per square meter area in bitter gourd ecosystem

Time	Post-monsoon (2018)						Summer (2019)						Monsoon (2019)					
	Bp	**Ch**	**Hs**	**Cs**	**Nc**	**Mean±SE**	**Bp**	**Ch**	**Hs**	**Cs**	**Nc**	**Mean±SE**	**Bp**	**Ch**	**Hs**	**Cs**	**Nc**	**Mean±SE**
T1	715	225	38	54	0	206.4±29.7	678	215	28	90	18	205.8±27.5	315	198	45	58	5	124.2±12.9
T2	891	378	94	99	21	296.6±35.9	805	449	50	172	44	304±32.4	425	356	128	128	9	209.2±17.4
T3	1105	418	161	175	123	396.4±41.2	912	434	174	250	234	400.8±30.1	515	484	256	230	32	303.4±19.9
T4	1147	441	266	274	103	446.2±40.9	1037	504	235	285	275	467.2±33.5	687	453	280	255	90	353±22.6
T5	1143	453	208	198	139	428.2±41.7	1011	474	156	320	190	430.2±34.7	761	384	119	326	118	341.6±26.3
T6	747	310	215	155	74	300.2±26.4	869	279	158	120	52	295.6±33.1	415	152	96	115	43	164.2±14.5

Bp- *Braunsapis picitarsis*, **Ch**- *Ceratina hieroglyphica*, **Hs**- *Halictus* sp., **Cs**- *Ceratina smaragdula*, **Nc**- *Nomia curvipes*

T1 – 6.00 AM to 7.00 AM **T2**- 7.00 AM to 8.00 AM **T3** - 8.00 AM to 9.00 AM **T4** - 9.00 AM to 10.00 AM

T5 - 10.00 AM to 11.00 AM **T6** - 11.00 AM to 12.00 PM

Table 16. Mean number solitary pollen bees per square meter area in oriental pickling melon ecosystem

Time	Post-monsoon (2018)						Summer (2019)						Monsoon (2019)					
	Ch	**Bp**	**Cs**	**Ha**	**Nc**	**Mean±SE**	**Ch**	**Bp**	**Cs**	**Ha**	**Nc**	**Mean±SE**	**Ch**	**Bp**	**Cs**	**Ha**	**Nc**	**Mean±SE**
T1	312	235	215	155	32	189.8±10.4	380	190	210	98	23	180.2±13.4	456	150	284	110	2	200.4±17.4
T2	350	280	330	213	57	246±11.8	448	278	224	112	115	235.4±13.8	512	175	335	145	13	236±19.2
T3	475	453	395	244	105	334.4±15.6	535	252	380	298	108	314.6±15.7	580	230	358	170	9	269.4±21.4
T4	561	430	437	289	130	369.4±16.4	715	328	530	315	45	360.4±25.2	735	345	460	162	17	343.8±27.6
T5	585	385	332	357	95	350.8±17.4	635	330	455	285	97	386.6±20.2	750	280	457	213	8	341.6±27.9
T6	402	110	214	128	34	177.6±14.1	339	109	305	54	35	168.4±14.3	318	215	310	76	15	186.8±13.6

Bp- *Braunsapis picitarsis*, **Ch**- *Ceratina hieroglyphica*, **Hs**- *Halictus* sp., **Cs**- *Ceratina smaragdula*, **Nc**- *Nomia curvipes*

T1 – 6.00 AM to 7.00 AM **T2**- 7.00 AM to 8.00 AM **T3** - 8.00 AM to 9.00 AM **T4** - 9.00 AM to 10.00 AM

T5 - 10.00 AM to 11.00 AM **T6** - 11.00 AM to 12.00 PM

4.2.2 Abundance of flower visitors at 100 per cent flowering in bitter gourd ecosystem during post-monsoon (2018)

The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in bitter gourd ecosystem during post-monsoon-2018 showed that, the stingless bee, *T. iridipennis* was the most abundant pollinator of bitter gourd ecosystem with a per cent abundance of 23.23, followed by *A. cerana* with a per cent abundance of 22.47. Among the solitary pollen bees recorded in the bitter gourd ecosystem, *B. picitarsis* was the most abundant with a per cent abundance of 22.26, followed by *C. hieoglyphica* (7.7 %) and *C. smaragdula* (3.05 %) (Table 17).

4.2.3 Abundance of flower visitors at 100 per cent flowering in bitter gourd ecosystem during summer-2019

The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in bitter gourd ecosystem during summer (2019) showed that, the stingless bee, *T. iridipennis* was the most abundant pollinator of bitter gourd ecosystem with a per cent abundance of 24.24, followed by *A. cerana* with a per cent abundance of 20.80. Among the solitary pollen bees recorded in the bitter gourd ecosystem, *B. picitarsis* was the most abundant with a per cent abundance of 19.08, followed by *C. hieoglyphica* (7.98 %) and *C. smaragdula* (6.93 %) (Table 18).

4.2.4 Abundance of flower visitors at 100 per cent flowering in bitter gourd ecosystem during monsoon-2019

The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in bitter gourd ecosystem during monsoon (2019) showed that, the stingless bee, *T. iridipennis* was the most abundant pollinator of bitter gourd ecosystem with a per cent abundance of 23.25, followed by *A. cerana* with a per cent abundance of 22.26. Among the solitary pollen bees recorded in the bitter gourd ecosystem, *B. picitarsis* was the most abundant with a per cent abundance of 15.88, followed by *C. smaragdula* (9.69 %), and *C. hieroglyphica* (9.38 %) (Table 19).

Table 17. Abundance of flower visitors at 100 % flowering in bitter gourd ecosystem (Post-monsoon-2018)

Pollinator	6 AM-7AM	7 AM-8 AM	8 AM-9 AM	9 AM-10 AM	10 AM-11 AM	11 AM-12 PM	12 PM-1 PM	1 PM-2 PM	2 PM-3 PM	3 PM-4 PM	4 PM-5 PM	5 PM-6 PM	Total	Mean	Abundance (%)
B. picitarsis	52	55	66	68	72	54	33	12	22	4	0	0	438	36.5	**22.26**
C. hieroglyphica	16	17	24	29	28	17	8	3	7	4	0	0	153	12.75	**7.77**
C. smaragdula	2	8	11	10	5	4	10	0	3	0	7	0	60	5.00	**3.05**
L. serenum	0	0	5	5	3	0	1	0	0	0	0	0	14	1.16	0.71
N. curvipes	0	0	3	7	5	5	2	0	1	0	0	0	23	1.91	1.16
Halictus sp.	2	5	11	10	12	6	6	3	0	1	0	0	56	4.66	2.84
A. cerana	10	29	45	56	68	69	54	56	23	15	11	6	442	36.83	**22.47**
A. florea	0	0	8	12	11	29	35	39	27	18	7	0	186	15.50	9.45
A. dorsata	0	0	0	2	11	3	15	0	0	3	1	0	35	2.91	1.77
T iridipennis	41	54	60	68	69	55	43	35	12	10	8	2	457	38.08	**23.23**
Acytolepis sp.	0	0	1	5	3	2	0	0	1	3	1	0	16	1.33	0.81
Eurema sp.	0	0	1	3	5	9	5	10	5	3	1	0	42	3.50	2.13
Tachytes sp.	0	0	0	0	2	2	0	0	8	5	1	0	18	1.50	0.91
Syritta sp.	0	0	2	1	1	2	0	0	1	2	1	0	10	0.83	0.50
E. macrops	0	0	0	0	0	3	0	3	11	0	0	0	17	1.41	0.86

Table 18. Abundance of flower visitors at 100 % flowering in bitter gourd ecosystem (summer-2019)

Pollinator	6 AM-7AM	7 AM-8 AM	8 AM-9 AM	9 AM-10 AM	10 AM-11 AM	11 AM-12 PM	12 PM-1 PM	1 PM-2 PM	2 PM-3 PM	3 PM-4 PM	4 PM-5 PM	5 PM-6 PM	Total	Mean	Abundance (%)
B. picitarsis	40	46	48	55	59	58	45	26	12	5	5	0	399	33.25	**19.08**
C. hieroglyphica	5	24	21	20	24	20	15	8	14	6	4	6	167	13.91	**7.98**
C. smaragdula	15	18	25	29	25	15	9	5	1	3	0	0	145	12.08	**6.93**
L. serenum	0	1	0	0	2	2	1	0	0	0	0	0	6	0.50	0.28
N. curvipes	1	0	2	8	8	8	5	4	0	2	0	0	38	3.16	1.81
Halictus sp.	7	16	19	18	20	15	9	0	0	7	3	0	114	9.50	5.45
A. cerana	2	39	44	58	65	60	59	38	27	20	15	8	435	36.25	**20.80**
A. florea	0	0	12	19	12	25	38	25	10	12	5	3	161	13.41	7.69
A. dorsata	0	0	4	0	2	7	0	0	3	1	1	2	20	1.66	0.95
T iridipennis	55	56	67	65	60	58	54	43	26	12	8	3	507	42.25	**24.24**
Acytolepis sp.	0	0	0	0	1	0	0	0	0	1	0	0	2	0.16	0.09
Eurema sp.	0	0	1	11	13	8	0	0	0	1	1	0	35	2.91	1.67
Tachytes sp.	0	0	0	0	3	0	0	0	3	7	2	0	15	1.25	0.71
Syritta sp.	0	0	0	2	6	3	0	0	0	0	0	0	11	0.91	0.52
E. macrops	0	0	0	6	4	3	2	0	11	8	2	0	36	3	1.72

Table 19. Abundance of flower visitors at 100 % flowering in bitter gourd ecosystem (Monsoon-2019)

Pollinator	6 AM-7AM	7 AM-8 AM	8 AM-9 AM	9 AM-10 AM	10 AM-11 AM	11 AM-12 PM	12 PM-1 PM	1 PM-2 PM	2 PM-3 PM	3 PM-4 PM	4 PM-5 PM	5 PM-6 PM	Total	Mean	Abundance (%)
B. picitarsis	25	34	33	44	37	28	22	12	10	11	3	0	259	21.58	**15.88**
C. hieroglyphica	10	22	20	27	32	22	8	10	2	0	0	0	153	12.75	**9.38**
C. smaragdula	5	14	21	25	29	21	17	12	5	7	2	0	158	13.16	**9.69**
L. serenum	0	0	0	0	0	2	0	0	0	0	0	0	2	0.18	0.12
N. curvipes	0	0	2	3	10	2	0	0	3	0	0	0	20	1.66	1.22
Halictus sp.	2	9	12	15	10	18	11	3	9	3	0	0	92	7.66	5.64
A. cerana	0	17	32	40	47	51	68	55	30	17	6	0	363	30.25	**22.26**
A. florea	0	0	4	10	15	26	34	37	27	5	0	0	158	13.16	9.69
A. dorsata	0	0	0	0	0	2	2	0	0	0	0	0	4	0.33	0.24
T iridipennis	26	38	40	48	56	59	55	33	11	10	3	0	379	31.58	**23.25**
Acytolepis sp.	0	0	0	1	0	2	0	0	0	1	0	0	4	0.33	0.24
Eurema sp.	0	0	0	2	5	1	0	0	2	2	1	0	13	1.08	0.79
Tachytes sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.00
Syritta sp.	0	0	0	0	1	3	3	0	0	0	0	0	7	0.58	0.42
E. macrops	0	0	0	5	2	2	0	0	2	7	0	0	18	1.50	1.10

4.2.5 Abundance of flower visitors at 100 per cent flowering in oriental pickling melon ecosystem (Post-monsoon-2018)

The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in oriental pickling melon ecosystem during post-monsoon-2018 showed that, the stingless bee, *T. iridipennis* was the most abundant pollinator of bitter gourd ecosystem with a per cent abundance of 21.55, followed by *A. cerana* with a per cent abundance of 17.87. AMong the solitary pollen bees recorded in the bitter gourd ecosystem, *B. picitarsis* was the most abundant with a per cent abundance of 14.79, followed by *C. hieroglyphica* (14.20 %) and *C. smaragdula* (5.19 %) (Table 20).

4.2.6 Abundance of flower visitors at 100 per cent flowering in oriental pickling melon ecosystem (summer-2019)

The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in oriental pickling melon ecosystem during summer (2019) showed that, the stingless bee, *T. iridipennis* was the most abundant pollinator of bitter gourd ecosystem with a per cent abundance of 18.57, followed by *A. cerana* with a per cent abundance of 14.94. Among the solitary pollen bees recorded in the bitter gourd ecosystem, *C. hieroglyphica* was the most abundant with a per cent abundance of 13.52, followed by *B. picitarsis* (11.80 %) and*, C. smaragdula* (11.61 %) (Table 21).

4.2.7 Abundance of flower visitors at 100 per cent flowering in oriental pickling melon ecosystem (monsoon-2019)

The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in oriental pickling melon ecosystem during mosoon-2019 showed that, the stingless bee, *T. iridipennis* was the most abundant pollinator of bitter gourd ecosystem with a per cent abundance of 21.99, followed by *A. cerana* with a per cent abundance of 18.25. Among the solitary pollen bees recorded in the bitter gourd ecosystem, *C. hieroglyphica* was the most abundant with a per cent abundance of 17.53, followed by *C. smaragdula* (14.38 %) and *B. picitarsis* (9.86 %) (Table 22).

Table 20. Abundance of flower visitors at 100 % flowering in oriental pickling melon ecosystem (Post-monsoon-2018)

Pollinator	6 AM-7AM	7 AM-8 AM	8 AM-9 AM	9 AM-10 AM	10 AM-11 AM	11 AM-12 PM	12 PM-1 PM	1 PM-2 PM	2 PM-3 PM	3 PM-4 PM	4 PM-5 PM	5 PM-6 PM	Total	Mean	Abundance (%)
C. hieroglyphica	29	48	45	55	52	43	23	9	5	0	0	0	309	25.75	**14.20**
B. picitarsis	32	40	56	58	55	32	28	12	9	0	0	0	322	26.83	**14.79**
C. smaragdula	10	21	14	20	22	7	9	10	0	0	0	0	113	9.41	**5.19**
Halictus sp.	0	8	13	17	16	14	6	0	0	8	0	0	82	6.83	3.76
N. curvipes	0	0	3	9	13	7	3	0	0	0	0	0	35	2.91	1.60
A. cerana	0	21	45	42	50	55	54	53	39	10	13	7	389	32.41	**17.87**
A. florea	0	0	5	23	31	35	44	40	25	9	13	0	225	18.75	10.34
A. dorsata	0	0	0	15	17	15	15	9	0	0	0	0	71	5.91	3.26
T iridipennis	37	35	56	53	57	61	60	43	19	21	17	10	469	39.08	**21.55**
Acytolepis sp.	0	0	1	1	5	2	0	0	0	0	0	0	9	0.75	0.41
Eurema sp.	0	0	7	3	11	15	8	0	0	0	0	0	44	3.66	2.02
Tachytes sp.	0	0	0	0	3	3	0	3	7	0	0	0	16	1.33	0.73
Syritta sp.	0	0	0	7	9	13	3	0	0	5	6	0	43	3.58	1.97
E. macrops	0	0	0	5	9	13	4	8	10	0	0	0	49	4.08	2.25

Table 21. Abundance of flower visitors at 100 % flowering in oriental pickling melon ecosystem (summer-2019)

Pollinator	6 AM-7AM	7 AM-8 AM	8 AM-9 AM	9 AM-10 AM	10 AM-11 AM	11 AM-12 PM	12 PM-1 PM	1 PM-2 PM	2 PM-3 PM	3 PM-4 PM	4 PM-5 PM	5 PM-6 PM	Total	Mean	Abundance (%)
C. hieroglyphica	28	45	49	52	58	43	29	19	18	9	4	0	354	29.5	**13.52**
B. picitarsis	33	40	45	55	40	36	24	20	11	5	0	0	309	25.75	**11.80**
C. smaragdula	35	38	45	55	61	35	20	9	6	0	0	0	304	25.33	**11.61**
Halictus sp.	0	3	15	19	22	11	14	10	7	5	0	0	106	8.83	4.05
N. curvipes	0	7	11	17	14	12	9	11	9	7	3	0	100	8.33	3.82
A. cerana	0	38	45	57	63	65	45	38	23	17	0	0	391	32.58	**14.94**
A. florea	0	0	9	24	35	40	45	27	17	5	0	0	202	16.83	7.718
A. dorsata	0	0	0	6	18	27	20	5	2	5	0	0	83	6.91	3.17
T iridipennis	45	58	66	65	70	49	43	32	25	11	13	9	486	40.50	**18.57**
Acytolepis sp.	0	0	0	6	4	1	1	3	1	0	0	0	16	1.33	0.61
Eurema sp.	0	0	7	12	15	20	28	7	10	3	0	1	103	8.58	3.93
Tachytes sp.	0	0	0	0	0	5	7	0	12	8	0	0	32	2.66	1.22
Syritta sp.	0	0	3	14	5	11	9	5	0	0	0	0	47	3.91	1.79
E. macrops	0	0	0	0	12	10	18	23	16	5	0	0	84	7.00	3.20

Table 22. Abundance of flower visitors at 100 % flowering in oriental pickling melon ecosystem (Monsoon-2019)

Pollinator	6 AM-7AM	7 AM-8 AM	8 AM-9 AM	9 AM-10 AM	10 AM-11 AM	11 AM-12 PM	12 PM-1 PM	1 PM-2 PM	2 PM-3 PM	3 PM-4 PM	4 PM-5 PM	5 PM-6 PM	Total	Mean	Abundance (%)
C. hieroglyphica	30	35	38	44	48	39	30	19	7	5	0	0	295	24.58	**17.53**
B. picitarsis	15	29	28	33	30	16	10	5	0	0	0	0	166	13.83	9.86
C. smaragdula	24	37	34	42	41	26	13	8	10	5	2	0	242	20.16	**14.38**
Halictus sp.	0	0	3	7	18	23	20	12	7	0	0	0	90	7.70	5.35
N. curvipes	0	0	0	7	10	5	5	2	0	3	0	0	32	2.66	1.90
A. cerana	0	25	47	45	52	48	39	20	18	9	4	0	307	25.58	**18.25**
A. florea	0	0	0	5	17	16	25	15	6	0	0	0	84	7.00	4.99
A. dorsata	0	0	0	4	7	12	4	0	0	0	0	0	27	2.25	1.60
T iridipennis	35	46	58	67	55	38	29	20	13	6	3	0	370	30.83	**21.99**
Acytolepis sp.	0	0	3	1	1	0	0	0	0	0	0	0	5	0.41	0.29
Eurema sp.	0	0	0	1	7	4	2	0	0	0	0	0	14	1.16	0.83
Tachytes sp.	0	0	0	0	0	1	0	5	9	2	0	0	17	1.41	1.01
Syritta sp.	0	0	1	0	3	0	0	0	0	0	0	0	4	0.33	0.23
E. macrops	0	0	0	0	0	2	1	8	13	5	0	0	29	2.41	1.72

4.2.8 Number of visit by pollinators in bitter gourd ecosystem at different per cent flowering during post-monsoon-2018

The visual counts on all flower visitors with respect to their number of visit at 25, 50, 75 and >90 per cent flowering stage in bitter gourd ecosystem during post-mosoon-2018 showed that, the highest number of visit during 25 per cent flowering was received from *T. iridipennis* (18.08±4.02) followed by *B. picitarsis* (14.25±5.05), *A. cerana* (12.75±4.92) and *C. hieroglyphica* (7.75±2.90) (Table 23). *T. iridipennis* was recorded as the pollinator with the highest number of visit (34.25±9.74) during the 50 per cent flowering stage of bitter gourd, followed by *B. picitarsis* (28.41±10.31), *A. cerana* (27.50±9.60), *A. florea* (13.58±4.96) and, *C. hieroglyphica* (10.33±4.46). When the bitter gourd flowers attained 75 per cent flowering stage, it received the highest number of visit from *T. iridipennis* with an average of 36.08±10.35, followed by *B. picitarsis* (32.5±10.97), *A. cerana* (31.41±10.36), *A. florea* (14.08±5.15) and *C. hieroglyphica* (12.16±4.87). *T. iridipennis* was recorded to give the highest number of average visit (38.08±10.94) to bitter gourd flowers during >90 per cent flowering stage, followed by *A. cerana* (36.83±10.59), *B. picitarsis* (36.5±12.47), *A. florea* (15.5±6.23) and, *C. hieroglyphica* (12.75±4.71). Thus, it was found that the number of visit by pollinators was increased with increase in flowering percentage during post-monsoon-2018.

4.2.9 Number of visit by pollinators in bitter gourd ecosystem at different per cent flowering during summer-2019

The visual counts on all flower visitors with respect to their number of visit at 25, 50, 75 and >90 per cent flowering stage in bitter gourd ecosystem during summer (2019) showed that, the highest number of visit during 25 per cent flowering was received from *T. iridipennis* (22.66±6.12) followed by *A. cerana* (17.08±4.58), *B. picitarsis* (13.08±4.49), and *C. hieroglyphica* (8.83±2.82) (Table 24). *T. iridipennis* was recorded as the pollinator with the highest number of visit (36.33±6.51) during the 50 per cent flowering stage of bitter gourd, followed by *A. cerana* (28.66±8.91), *B. picitarsis* (27.5±7.71), *C. hieroglyphica* (11.25±4.64) and, *A. florea* (10.25±4.65) .When the bitter gourd flowers attained 75 per cent flowering stage, it received the highest number of visit from *T. iridipennis* with an average of 40.25±9.23, followed

by *A. cerana* (35.41±9.64), *B. picitarsis* (32.75±9.86), *C. hieroglyphica* (13.16±4.62) and, *A. florea* (12.75±5.02). *T. iridipennis* was recorded to give the highest number of average visit (42.25±10.51) to bitter gourd flowers during >90 per cent flowering stage, followed by *A. cerana* (36.25±9.74), *B. picitarsis* (33.25±10.01), *C. hieroglyphica* (13.91±3.48) and, *A. florea* (13.41±5.15). The number of visit by pollinators was comparatively higher in summer (2019) as compared to that of post-monsoon-2018 and, it was found that the number of visit by all pollinators increased with increase in flowering percentage.

4.2.10 Number of visit by pollinators in bitter gourd ecosystem at different per cent flowering during monsoon-2019

The visual counts on all flower visitors with respect to their number of visit at 25, 50, 75 and >90 per cent flowering stage in bitter gourd ecosystem during monsoon-2019 showed that, the highest number of visit during 25 per cent flowering was received from *T. iridipennis* (13.19±3.76) followed by *B. picitarsis* (8.75±3.05), *A. cerana* (7.08±2.85), and *C. hieroglyphica* (4.5±1.76) (Table 25). *T. iridipennis* was recorded as the pollinator with the highest number of visit (21.58±4.95) during the 50 per cent flowering stage of bitter gourd, followed by *A. cerana* (20.5±5.67), *B. picitarsis* (19.5±0.73) and, *C. hieroglyphica* (10.16±3.78).When the bitter gourd flowers attained 75 per cent flowering stage, it received the highest number of visit from *T. iridipennis* with an average of 27.91±8.01, followed by *A. cerana* (26.58±7.35), *B. picitarsis* (20.41±6.91) and, *C. smaragdula* (13.41±5.11). *T. iridipennis* was recorded to give the highest number of average visit (31.58±9.54) to bitter gourd flowers during >90 per cent flowering stage, followed by *A. cerana* (30.25±10.10), *B. picitarsis* (21.58±6.36), *A. florea* (13.16±6.34) and, *C. smaragdula* (13.16±4.26). Though the number of bee visit was comparatively lower during monsoon-2019, it was found that the number of visit by all pollinators increased with increase in flowering percentage.

Table 23. Average number of visit by pollinators in bitter gourd ecosystem at different per cent flowering during post-monsoon-2018

Pollinators	Mean±SE (N=5)			
	25% flowering	50%flowering	75%flowering	>90% flowering
B. picitarsis	**14.25±5.05**	**28.41±10.31**	**32.5±10.97**	**36.5±12.47**
C. hieroglyphica	**7.75±2.90**	**10.33±4.46**	**12.16±4.87**	**12.75±4.71**
C. smaragdula	2.16±0.89	3.58±1.34	4.83±1.92	5±1.84
L. serenum	0.75±0.83	0.75±0.83	0.83±0.49	1.16±0.89
N. curvipes	0.91±0.69	1.08±0.90	1.75±0.97	1.91±1.11
Halictus sp.	3.33±1.49	4.66±1.54	4.41±1.87	4.66±1.97
A. cerana	**12.75±4.92**	**27.50±9.60**	**31.41±10.36**	**36.83±10.59**
A. florea	**6±2.45**	**13.58±4.96**	**14.08±5.15**	**15.5±6.23**
A. dorsata	0.08±0.12	0.91±0.88	0.83±0.91	2.91±2.20
T. iridipennis	**18.08±4.02**	**34.25±9.74**	**36.08±10.35**	**38.08±10.94**
Acytolepis sp.	0.08±0.12	0.41±0.40	1.08±0.69	1.33±0.72
Eurema sp.	0.33±0.29	0.83±0.53	2.58±1.29	3.5±1.53
Tachytes sp.	0.00	0.16±0.17	0.83±0.78	1.5±1.13
Syritta sp.	0.16±0.25	0.50±0.44	0.83±0.37	0.83±0.37
E. macrops	0.00	0.00	0.5±0.44	1.41±1.44

Table 24. Average number of visit by pollinators in bitter gourd ecosystem at different flowering per cent during summer-2019

Pollinators	Mean±SE (N=5)			
	25% flowering	**50%flowering**	**75%flowering**	**>90% flowering**
B. picitarsis	**13.08±4.49**	**27.5±7.71**	**32.75±9.86**	**33.25±10.01**
C. hieroglyphica	**8.83±2.82**	**11.25±4.64**	**13.16±4.62**	**13.91±3.48**
C. smaragdula	7.16±3.89	10.66±3.96	11.58±3.18	12.08±4.72
L. serenum	0.00	0.66±0.47	0.50±0.30	0.50±0.35
N. curvipes	0.41±0.35	0.58±0.44	2.75±1.55	3.16±1.48
Halictus sp.	3.41±1.66	5.75±2.28	6.08±3.52	9.50±3.49
A. cerana	**17.08±4.58**	**28.66±8.91**	**35.41±9.64**	**36.25±9.74**
A. florea	**5.41±3.13**	**10.25±4.65**	**12.75±5.02**	**13.41±5.15**
A. dorsata	0.00	2.58±1.99	3.08±2.16	1.66±0.95
T. iridipennis	**22.66±6.12**	**36.33±6.51**	**40.25±9.23**	**42.25±10.51**
Acytolepis sp.	0.08±0.12	0.91±0.61	0.83±0.49	0.16±0.17
Eurema sp.	0.25±0.27	1.08±0.69	1.66±1.39	2.91±2.15
Tachytes sp.	0.00	0.91±0.72	0.66±0.64	1.25±0.97
Syritta sp.	0.18±0.18	0.58±0.40	0.75±0.78	0.91±0.84
E. macrops	0.00	0.75±0.47	1.08±0.84	3±1.62

Table 25. Average number of visit by pollinators in bitter gourd ecosystem at different flowering per cent during monsoon-2019

Pollinators	Mean±SE (N=5)			
	25% flowering	50%flowering	75%flowering	>90% flowering
B. picitarsis	**8.75±3.05**	**19.5±0.73**	**20.41±6.91**	**21.58±6.36**
C. hieroglyphica	**4.5±1.76**	10.16±3.78	12.66±4.30	12.75±5.11
C. smaragdula	3.75±1.77	**9.66±3.02**	**13.41±5.11**	**13.16±4.26**
L. serenum	0.00	0.45±0.46	0.45±0.46	0.18±0.26
N. curvipes	0.00	1.91±1.15	1.75±1.08	1.66±1.29
Halictus sp.	0.58±0.40	5.41±2.02	6.83±3.18	7.66±2.67
A. cerana	**7.08±2.85**	**20.5±5.67**	**26.58±7.35**	**30.25±10.10**
A. florea	1.16±0.82	9.08±4.10	12.16±5.33	13.16±6.34
A. dorsata	0.08±0.12	0.58±0.55	0.33±0.22	0.33±0.34
T. iridipennis	**13.19±3.76**	**21.58±4.95**	**27.91±8.01**	**31.58±9.54**
Acytolepis sp.	0.16±0.17	0.50±0.40	0.08±0.12	0.33±0.29
Eurema sp.	0.08±0.12	1.08±0.67	1.08±0.67	1.08±0.67
Tachytes sp.	0.00	0.25±0.27	0.00	0.00
Syritta sp.	0.16±0.17	0.00	0.25±0.20	0.58±0.52
E. macrops	0.08±0.12	0.08±0.12	0.5±0.44	1.50±1.03

4.2.11 Number of visit by pollinators in oriental pickling melon ecosystem at different per cent flowering during post-monsoon-2018

The visual counts on all flower visitors with respect to their number of visit at 25, 50, 75 and >90 per cent flowering stage in oriental pickling melon ecosystem during post-monsoon-2018 showed that, the highest number of visit during 25 per cent flowering was received from *T. iridipennis* (18.83±3.92) followed by *C. hieroglyphica* (13.3±4.44), *B. picitarsis* (12±4.08), *A. cerana* (10.41±3.75) and, *A. florea* (4.25±2.34) (Table 26). *T. iridipennis* was recorded as the pollinator with the highest number of visit (30.33±8.84) during the 50 per cent flowering stage of oriental pickling melon, followed by *A. cerana* (25.16±9.14), *C. hieroglyphica* (22.08±8.01), *B. picitarsis* (19.75±8.64) and, *A. florea* (11.25±4.58). When the oriental pickling melon flowers attained 75 per cent flowering stage, it received the highest number of

visit from *T. iridipennis* with an average of 35.33±8.17, followed by *A. cerana* (30.75±9.70), *C. hieroglyphica* (24.91±9.20), *B. picitarsis* (22.33±10.13) and, *A. florea* (16.66±5.93). *T. iridipennis* was recorded to give the highest number of average visit (39.08±8.33) to oriental pickling melon flowers during >90 per cent flowering stage, followed by *A. cerana* (32.41±9.25), *B. picitarsis* (26.83±10.03), *C. hieroglyphica* (25.75±9.93) and, *A. florea* (18.75±7.30). The number of visit by pollinators increased with increase in flowering percentage during post-mosoon 2018.

4.2.12 Number of visit by pollinators in oriental pickling melon ecosystem at different per cent flowering during summer-2019

The visual counts on all flower visitors with respect to their number of visit at 25, 50, 75 and >90 per cent flowering stage in oriental pickling melon ecosystem during summer (2019) showed that, the highest number of visit during 25 per cent flowering was received from *T. iridipennis* (25.08±07.30) followed by *C. hieroglyphica* (17.00±06.90), *A. cerana* (16.41±06.49) and, *C. smaragdula* (14.25±06.52) (Table 27). *T. iridipennis* was recorded as the pollinator with the highest number of visit (33.75±07.48) during the 50 per cent flowering stage of oriental pickling melon, followed by *A. cerana* (28.00±07.46), *C. hieroglyphica* (26.58±09.44) and, *B. picitarsis* (24.75±08.72). When the oriental pickling melon flowers attained 75 per cent flowering stage, it received the highest number of visit from *T. iridipennis* with an average of 36.50±10.46, followed by *A. cerana* (30.50±10.43), *C. hieroglyphica* (28.08±09.18) and, *B. picitarsis* (25.08±08.38). *T. iridipennis* was recorded to give the highest number of average visit (40.50±09.96) to oriental pickling melon flowers during >90 per cent flowering stage, followed by *A. cerana* (32.58±10.83), *C. hieroglyphica* (29.50±08.84) and, *B. picitarsis* (25.75±08.31). The number of visit by pollinators increased with increase in flowering percentage during summer (2019).

4.2.13 Number of visit by pollinators in oriental pickling melon ecosystem at different per cent flowering during monsoon-2019

The visual counts on all flower visitors with respect to their number of visit at 25, 50, 75 and, >90 per cent flowering stage in oriental pickling melon ecosystem during monsoon (2019) showed that, the highest number of visit during 25 per cent

flowering was received from *T. iridipennis* (13.41±04.74) followed by *C. hieroglyphica* (11.16±04.36), *C. smaragdula* (09.00±03.41) and *A. cerana* (08.33±03.83) (Table 28). *T. iridipennis* was recorded as the pollinator with the highest number of visit (26.58±08.27) during the 50 per cent flowering stage of oriental pickling melon, followed by *A. cerana* (22.16±06.27), *C. hieroglyphica* (17.75±06.38) and, *C. smaragdula* (17.16±05.89). When the oriental pickling melon flowers attained 75 per cent flowering stage, it received the highest number of visit from *T. iridipennis* with an average of 29.08±08.44, followed by *A. cerana* (24.16±07.60), *C. smaragdula* (22.41±07.83) and, *C. hieroglyphica* (21.33±07.08). *T. iridipennis* was recorded to give the highest number of average visit (30.83±10.18) to oriental pickling melon flowers during >90 per cent flowering stage, followed by *A. cerana* (25.58±08.89), *C. hieroglyphica* (24.58±07.87) and *C. smaragdula* (20.16±07.01). The number of visit by *C. smaragdula* in the oriental pickling melon ecosystem was comparatively higher in monsoon (2019) as compared to the other seasons. The number of visit by all pollinators also increased with increase in flowering percentage during monsoon (2019).

Table 26. Average number of visit by pollinators in oriental pickling melon ecosystem at different flowering per cent during post-monsoon-2018

Pollinators	Mean±SE (N=5)			
	25% flowering	50%flowering	75%flowering	>90% flowering
C. hieroglyphica	**13.3±4.44**	**22.08±8.01**	**24.91±9.20**	**25.75±9.93**
B. picitarsis	**12±4.08**	**19.75±8.64**	**22.33±10.13**	**26.83±10.03**
C. smaragdula	4.08±1.75	7.08±2.20	8.50±4.25	9.41±3.78
Halictus sp.	0.16±0.25	3±1.82	6.66±2.84	6.83±3.05
N. curvipes	0.00	0.16±0.25	1.83±1.18	2.91±1.97
A. cerana	**10.41±3.75**	**25.16±9.14**	**30.75±9.70**	**32.41±9.25**
A. florea	**4.25±2.34**	**11.25±4.58**	**16.66±5.93**	**18.75±7.30**
A. dorsata	0.25±0.38	3.25±2.20	5.41±2.85	5.91±3.37
T. iridipennis	**18.83±3.92**	**30.33±8.84**	**35.33±8.17**	**39.08±8.33**
Acytolepis sp.	0.91±0.58	0.58±0.48	1.25±1.01	0.75±0.66
Eurema sp.	0.66±0.72	1.83±1.04	3.33±1.71	3.66±2.36
Tachytes sp.	0.00	2.08±1.13	1.16±0.82	1.33±0.99
Syritta sp.	0.00	1.08±0.74	4.58±2.63	3.58±1.97
E. macrops	0.00	2.91±1.54	3.25±2.44	4.08±2.15

Table 27. Average number of visit by pollinators in oriental pickling melon ecosystem at different flowering per cent during summer-2019

Pollinators	Mean±SE (N=5)			
	25% flowering	50%flowering	75%flowering	>90% flowering
C. hieroglyphica	**17.00±06.90**	**26.58±09.44**	**28.08±09.18**	**29.50±08.84**
B. picitarsis	14.08±05.10	**24.75±08.72**	**25.08±08.38**	**25.75±08.31**
C. smaragdula	**14.25±06.52**	22.66±10.06	23.16±09.44	25.33±09.98
Halictus sp.	03.25±01.98	08.50±03.79	08.66±03.61	08.83±03.39
N. curvipes	02.16±01.49	07.33±03.64	08.00±02.71	08.83±02.36
A. cerana	**16.41±06.49**	**28.00±07.46**	**30.50±10.43**	**32.58±10.83**
A. florea	09.41±04.97	14.08±05.90	16.75±07.44	16.83±07.56
A. dorsata	00.00±00.00	04.25±02.43	04.91±02.82	06.91±04.19
T. iridipennis	**25.08±07.30**	**33.75±07.48**	**36.50±10.46**	**40.50±09.96**
Acytolepis sp.	00.50±00.52	01.00±00.83	02.16±01.34	01.33±00.88
Eurema sp.	00.41±00.40	04.91±02.65	04.58±02.43	08.58±03.99
Tachytes sp.	00.25±00.27	00.66±00.72	00.66±00.90	02.66±01.89
Syritta sp.	00.58±00.67	03.50±02.34	04.41±02.36	03.91±02.22
E. macrops	01.16±01.27	04.91±03.28	05.33±02.83	07.00±03.78

Table 28. Average number of visit by pollinators in oriental pickling melon ecosystem at different flowering per cent during monsoon-2019

Pollinators	Mean±SE (N=5)			
	25% flowering	50%flowering	75%flowering	>90% flowering
C. hieroglyphica	**11.16±04.36**	**17.75±06.38**	**21.33±07.08**	**24.58±07.87**
B. picitarsis	07.16±03.41	14.58±05.07	15.08±06.04	13.83±05.91
C. smaragdula	**09.00±03.41**	**17.16±05.89**	**22.41±07.83**	**20.16±07.01**
Halictus sp.	01.16±01.02	03.16±01.63	03.91±02.17	07.05±03.88
N. curvipes	00.00±00.00	02.16±01.49	03.00±01.91	02.66±01.51
A. cerana	**08.33±03.83**	**22.16±06.27**	**24.16±07.60**	**25.58±08.89**
A. florea	01.33±01.11	04.08±02.51	05.75±03.25	07.00±03.97
A. dorsata	00.00±00.00	01.91±01.58	02.41±01.77	02.25±01.75
T. iridipennis	**13.41±04.74**	**26.58±08.27**	**29.08±08.44**	**30.83±10.18**
Acytolepis sp.	00.00±00.00	00.33±00.39	00.75±00.47	00.41±00.40
Eurema sp.	00.00±00.00	01.00±00.71	01.41±00.79	01.16±00.98
Tachytes sp.	00.00±00.00	00.00±00.00	00.41±00.44	01.41±01.25
Syritta sp.	00.00±00.00	00.25±00.27	00.50±00.40	00.33±00.39
E. macrops	00.25±00.27	01.00±00.87	01.25±00.89	02.41±01.87

4.3. Studying the nesting preferences of solitary pollen bees

Artificial nesting sites with varied nest hole diameters (2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 8, 9, 9.5, 10, 11, 12, 12.5, 13, 14 and 15mm) were made with materials *i.e*., wood and naturally available tubular plant materials (reeds, bamboos and dry hollow twigs of plants) (Plate 2A & 2B). The nesting blocks were placed at 10, 100 and 250 meters away from the cucurbit ecosystem. The per cent nesting by all arthropod nesters as well as the solitary pollen bees were recorded at monthly intervals from 2019 to 2021, to understand the effect of various treatments on per cent nesting.

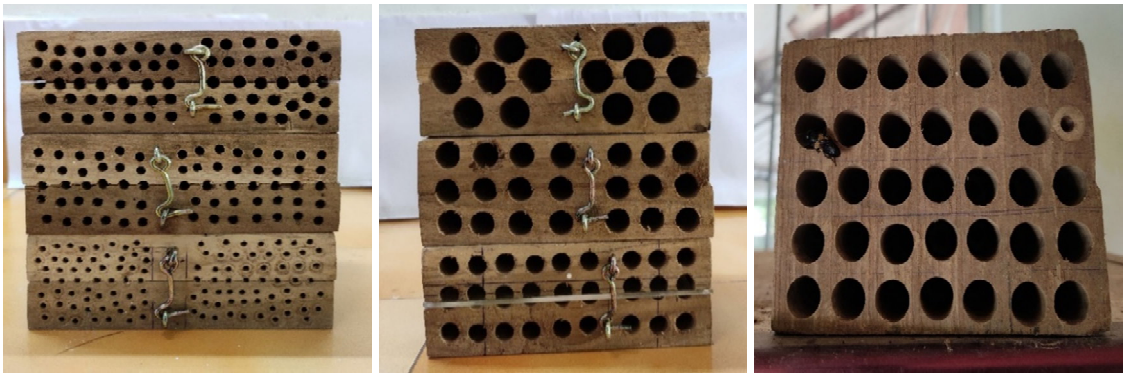


Plate 2A) Artficial nests made of wooden blocks

Plate 2B) Artificial nests made of naturally available tubular plant materials

4.3.1. Preference of nest hole diameters towards the nesting of arthropod nesters

The per cent nest occupancy of all arthropod nesters in the year 2019 showed that, hole sizes influenced the per cent occupancy of all arthropod nesters significantly (Table 29). Several arthropod species were found to occupy nest hole diameters *viz.*, 4 mm, 5 mm, 6 mm, 7 mm, 8 mm, 9 mm, 9.5 mm, 10 mm, 12 mm, 14 mm and 15 mm. The nest hole diameters showed specificity to arthropod species such as, *Dasyproctus* sp. preferred a nest hole diameter of 4 mm whereas *C. bengalense* preferred a hole diameter of 5 mm and 6 mm. Though the potter wasp *R. brunneum* mostly preferred a nest hole diameter of 10 mm, they were also found occupied in 7 mm and 8 mm nest hole diameters. Several ants and spider species were found to occupy the nest hole diameters *viz.*, 8, 9, 9.5, 10, 12, 13, 14 and 15 mm.

The solitary bee nesters were hardly attracted to the artificial nesting sites in the year 2019 except the megachilid bee species *M. disjuncta* (Table 30) which was found occupied in the nest hole diameter of 10 mm mostly. Though they were also found to be attracted to the nest hole diameter of 12 mm.

The per cent nest occupancy of all arthropod nesters in the year 2020 (Table 31) showed that, arthropod nesters occupying the artificial nesting sites other than ants and spiders were *C. bengalense*, *Dasyproctus* sp. and *R. brunneum*. *Dasyproctus* sp. preferred the nest diameter with 4 mm whereas the *C. bengalense* preferred 5mm and 6 mm nest diameters. *R. brunneum* was found occupied in the nest diameter of 7, 8 and 10 mm. Ants and spiders occupied in nests made with tubular plant materials with diameters *viz.*, 8, 9, 9.5, 10, 11, 12, 12.5, 13, 14 and 15 mm.

The per cent occupancy of solitary pollen bee nesters in the year 2020 showed that (Table 32), the solitary megachilid bee, *M. disjuncta* was attracted to nest hole diameters of 7, 10 and 12 mm. Whereas *Megachile* sp. was found occupied in the nest hole diameters of 6, 9 and 9.5 mm. The solitary bee species other than the megachilid bees were attracted to nest hole sizes *viz*., 2.5 and 3 mm.

The per cent occupancy of all arthropod nesters in the year 2021 showed that (Table 33), the nest hole diameters *viz*., 4, 4.5, 5, 6, 7, 8, 9, 9.5, 10, 12, 13, 14 and 15 mm were continuously occupied by different arthropod species. *Dasyproctus* sp. was specifically found occupied in nest hole diameter of 4 mm whereas *C. bengalense* were found in 5 mm and 6 mm diameters. *R. brunneum* was seldom occupied the nest

in the year 2021 as compared to 2019 and 2020. Observations on solitary pollen bee nesting during 2021 (Table 34) showed increased per cent occupancy in the artificial nest sites. The solitary megachilid bee, *M. disjuncta* was found occupied in nest hole diameters of 7, 10 and 12 mm. Whereas, solitary pollen bee species such as *B. picitarsis*, *C. smaragdula* and *C. hieroglyphica* were attracted to nest hole diameters of 2.5, 3 and 3.5 m.

Table 29. Effect of hole size diameter (A), nesting substrate (B) and distances (C) in per cent nest occupancy of all arthropods nesters in 2019

ANOVA	DF	January		February		March		April		May		June		July		August		September		October		November		December	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Factor A	19	4.61	0.00	4.23	0.00	2.40	0.00	3.12	0.00	1.60	0.05	5.96	0.00	2.81	0.00	3.08	0.00	2.28	0.00	3.91	0.00	4.07	0.00	4.26	0.00
Factor B	1	2.03	0.15	3.01	0.08	1.11	0.29	0.02	0.86	0.25	0.61	22.52	0.00	5.43	0.02	2.61	0.10	0.49	0.48	0.36	0.54	9.30	0.00	16.64	0.00
Factor C	2	2.35	0.09	3.59	0.02	0.21	0.81	0.22	0.79	0.59	0.55	4.84	0.00	3.21	0.04	0.09	0.90	2.88	0.05	0.52	0.59	0.20	0.81	2.27	0.10
A×B	19	3.78	0.00	1.63	0.04	1.68	0.03	2.42	0.00	0.97	0.48	4.90	0.00	2.75	0.00	3.92	0.00	1.19	0.25	0.77	0.73	4.80	0.00	4.51	0.00
A×C	38	4.07	0.00	1.41	0.06	1.72	0.00	1.10	0.31	0.81	0.77	1.63	0.01	1.73	0.00	1.55	0.02	1.60	0.01	0.94	0.57	0.93	0.57	1.26	0.14
B×C	2	9.52	0.00	3.59	0.02	1.19	0.30	0.22	0.79	3.26	0.03	5.63	0.00	2.42	0.09	2.61	0.07	2.00	0.13	2.26	0.10	2.47	0.08	2.27	0.10
A×B×C	38	4.05	0.00	1.90	0.00	1.86	0.00	0.61	0.96	1.23	0.17	2.34	0.00	1.63	0.01	1.56	0.02	0.59	0.97	1.44	0.05	0.81	0.76	1.20	0.20

Factor A – hole size (n=20)

Factor B – materials (n=2)

Factor C – distance from the field (n=3)

Table 30. Effect of hole size diameter (A), nesting substrate (B) and distances (C) in per cent nest occupancy of solitary pollen bee nesters in 2019

ANOVA	DF	January		February		March		April		May		June		July		August		September		October		November		December	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Factor A	19	NA	NA	1.00	0.46	1.00	0.46	1.00	0.46	4.00	0.00	0.94	0.52	NA	NA	NA	NA	NA	NA	1.00	0.4	1.00	0.46	NA	NA
Factor B	1	NA	NA	1.00	0.31	1.00	0.31	1.00	0.31	4.00	<0.05	2.00	0.15	NA	NA	NA	NA	NA	NA	1.00	0.31	1.00	0.31	NA	NA
Factor C	2	NA	NA	1.00	0.36	1.00	0.36	1.00	0.36	4.00	<0.02	2.00	0.13	NA	NA	NA	NA	NA	NA	1.00	0.36	1.00	0.36	NA	NA
A×B	19	NA	NA	1.00	0.46	1.00	0.46	1.00	0.46	4.00	0.00	0.94	0.52	NA	NA	NA	NA	NA	NA	1.00	0.46	1.00	0.46	NA	NA
A×C	38	NA	NA	1.00	0.47	1.00	0.47	1.00	0.47	4.00	0.00	0.94	0.56	NA	NA	NA	NA	NA	NA	1.00	0.47	1.00	0.47	NA	NA
B×C	2	NA	NA	1.00	0.36	1.00	0.36	1.00	0.36	4.00	<0.02	2.00	0.13	NA	NA	NA	NA	NA	NA	1.00	0.36	1.00	0.36	NA	NA
A×B×C	38	NA	NA	1.00	0.47	1.00	0.47	1.00	0.47	4.00	0.00	0.94	0.56	NA	NA	NA	NA	NA	NA	1.00	0.47	1.00	0.47	NA	NA

Factor A – hole size (n=20)

Factor B – materials (n=2)

Factor C – distance from the field (n=3)

Table 31. Effect of hole size diameter (A), nesting substrate (B) and distances (C) in per cent nest occupancy of all arthropods in 2020

ANOVA	DF	January		February		March		April		May		June		July		August		September		October		November		December	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Factor A	19	1.42	0.11	0.92	0.54	1.99	0.00	2.96	0.00	3.89	0.00	2.18	0.00	9.29	0.00	56.32	0.00	8.62	0.00	3.70	0.00	2.09	0.00	3.60	0.00
Factor B	1	0.24	0.62	0.39	0.53	8.14	0.00	6.06	0.01	1.81	0.17	0.31	0.57	7.51	0.00	104.86	0.00	5.29	0.02	3.02	0.08	9.60	0.00	24.27	0.00
Factor C	2	5.04	0.00	0.69	0.49	0.90	0.40	1.93	0.14	0.10	0.89	2.09	0.12	1.75	0.17	5.93	0.00	9.14	0.00	0.32	0.72	0.60	0.54	0.98	0.37
A×B	19	2.01	0.00	1.24	0.22	1.99	0.00	3.24	0.00	4.38	0.00	1.46	0.10	9.49	0.00	52.47	0.00	2.51	0.00	2.64	0.00	1.31	0.17	3.71	0.00
A×C	38	1.37	0.08	0.90	0.62	0.79	0.79	1.37	0.07	1.98	0.00	1.19	0.21	2.74	0.00	21.29	0.00	3.43	0.00	0.89	0.65	0.88	0.67	1.99	0.00
B×C	2	6.71	0.00	2.49	0.08	0.90	0.40	4.59	0.01	4.80	0.00	7.53	0.00	3.63	0.02	188.90	0.00	19.46	0.00	6.64	0.00	1.80	0.16	1.93	0.14
A×B×C	38	1.47	0.04	0.81	0.77	0.79	0.79	1.23	0.17	1.74	0.00	1.56	0.02	2.64	0.00	23.675	0.00	6.48	0.00	1.86	0.00	1.51	0.03	2.10	0.00

Factor A – hole size (n=20)

Factor B – materials (n=2)

Factor C – distance from the field (n=3)

Table 32. Effect of hole size diameter (A), nesting substrate (B) and distances (C) in per cent nest occupancy of solitary pollen bees in 2020

ANOVA	DF	January		February		March		April		May		June		July		August		September		October		November		December	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Factor A	19	4.00	0.00	1.00	0.46	1.00	0.46	0.94	0.52	3.03	0.00	NA	NA	1.00	0.46	10.14	0.00	4.00	0.00	0.94	0.52	0.89	0.58	2.39	0.00
Factor B	1	4.00	0.04	1.00	0.31	1.00	0.31	2.00	0.15	3.03	0.08	NA	NA	1.00	0.31	19.63	0.00	4.00	0.04	2.00	0.15	0.33	0.56	4.50	0.03
Factor C	2	4.00	0.01	1.00	0.36	1.00	0.36	2.00	0.13	3.03	0.05	NA	NA	1.00	0.36	19.63	0.00	4.00	0.01	2.00	0.13	3.00	0.05	4.50	0.01
A×B	19	4.00	0.00	1.00	0.46	1.00	0.46	0.94	0.52	3.03	0.00	NA	NA	1.00	0.46	10.14	0.00	4.00	0.00	0.94	0.52	1.03	0.42	2.39	0.01
A×C	38	4.00	0.00	1.00	0.47	1.00	0.47	0.94	0.56	3.03	0.00	NA	NA	1.00	0.47	10.14	0.00	4.00	0.00	0.94	0.56	0.89	0.64	2.39	0.00
B×C	2	4.00	0.01	1.00	0.36	1.00	0.36	2.00	0.13	3.03	0.05	NA	NA	1.00	0.36	19.63	0.00	4.00	0.01	2.00	0.13	0.33	0.71	4.50	0.01
A×B×C	38	4.00	0.00	1.00	0.47	1.00	0.47	0.94	0.56	3.03	0.00	NA	NA	1.00	0.47	10.14	0.00	4.00	0.00	0.94	0.56	1.03	0.42	2.39	0.00

Factor A – hole size (n=20)

Factor B – materials (n=2)

Factor C – distance from the field (n=3)

Table 33. Effect of hole size diameter (A), nesting substrate (B) and distances (C) in per cent nest occupancy of all arthropods in 2021

ANOVA	DF	January		February		March		April		May		June		July		August		September		October		November		December	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Factor A	19	2.15	0.00	2.87	0.00	1.79	0.02	3.59	0.00	2.20	0.00	10.43	0.00	2.81	0.00	3.46	0.00	5.99	0.00	8.79	0.00	4.23	0.00	4.12	0.00
Factor B	1	4.07	0.04	0.28	0.59	2.68	0.10	3.16	0.07	1.50	0.22	0.69	0.40	1.07	0.30	0.17	0.67	4.00	0.04	22.19	0.00	14.86	0.00	12.11	0.00
Factor C	2	1.59	0.20	2.65	0.07	2.38	0.09	5.16	0.00	0.16	0.84	3.81	0.02	0.17	0.84	20.52	0.00	4.72	0.00	6.17	0.00	16.77	0.00	0.92	0.39
A×B	19	2.68	0.00	3.15	0.00	2.50	0.00	5.62	0.00	2.55	0.00	2.30	0.00	1.91	0.01	5.03	0.00	7.71	0.00	7.79	0.00	5.70	0.00	4.57	0.00
A×C	38	2.02	0.00	2.65	0.00	1.98	0.00	5.30	0.00	1.04	0.40	3.43	0.00	1.41	0.06	3.68	0.00	6.34	0.00	8.85	0.00	4.96	0.00	1.98	0.00
B×C	2	0.59	0.55	6.51	0.00	2.72	0.06	5.98	0.00	3.50	0.03	2.08	0.12	7.57	0.00	0.32	0.72	1.52	0.21	2.01	0.13	0.67	0.51	2.22	0.11
A×B×C	38	2.07	0.00	3.06	0.00	1.97	0.00	5.26	0.00	0.86	0.69	1.96	0.00	1.86	0.00	4.74	0.00	6.51	0.00	8.86	0.00	6.02	0.00	1.91	0.00

Factor A – hole size (n=20)

Factor B – materials (n=2)

Factor C – distance from the field (n=3)

Table 34. Effect of hole size diameter (A), nesting substrate (B) and distances (C) in per cent nest occupancy of solitary pollen bees in 2021

ANOVA	DF	January		February		March		April		May		June		July		August		September		October		November		December	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Factor A	19	2.39	0.00	1.53	0.07	2.42	0.00	11.13	0.00	3.03	0.00	6.56	0.00	1.34	0.15	3.08	0.00	31.80	0.00	12.76	0.00	9.65	0.00	2.39	0.00
Factor B	1	4.50	0.03	0.00	0.98	7.49	0.00	16.82	0.00	3.03	0.08	8.40	0.00	0.00	1.00	3.41	0.06	40.33	0.00	20.22	0.00	11.26	0.00	4.50	0.03
Factor C	2	4.50	0.01	3.23	0.04	7.49	0.00	18.24	0.00	3.03	0.05	16.54	0.00	6.44	0.00	9.43	0.00	40.33	0.00	28.38	0.00	29.60	0.00	4.50	0.01
A×B	19	2.39	0.00	1.70	0.03	2.42	0.00	9.29	0.00	3.03	0.00	6.99	0.00	1.68	0.03	3.39	0.00	31.80	0.00	13.19	0.00	10.61	0.00	2.39	0.00
A×C	38	2.39	0.00	1.53	0.03	2.42	0.00	8.79	0.00	3.03	0.00	6.56	0.00	1.34	0.09	3.08	0.00	31.80	0.00	12.76	0.00	9.65	0.00	2.39	0.00
B×C	2	4.50	0.01	0.00	0.99	7.49	0.00	13.16	0.00	3.03	0.05	8.40	0.00	0.00	1.00	3.41	0.03	40.33	0.00	20.22	0.00	11.26	0.00	4.50	0.01
A×B×C	38	2.39	0.00	1.70	0.00	2.42	0.00	6.92	0.00	3.03	0.00	6.99	0.00	1.68	0.01	3.39	0.00	31.80	0.00	13.19	0.00	10.61	0.00	2.39	0.00

Factor A – hole size (n=20)

Factor B – materials (n=2)

Factor C – distance from the field (n=3)

4.3.2. Preference of nesting materials towards the nesting of arthropod nesters

The per cent occupancy by arthropod nesters including the solitary pollen bees showed preference towards the nesting substrates. In the present study, solitary pollen bee nesters except the megachilid bees showed preference towards the naturally available tubular-hollow plant materials, whereas species specific preference towards the nesting material was observed in megachilid bee species. Every arthropod species other than ants and spiders were only attracted to the nests made of wooden blocks which were closed at one end. Ants and spiders were only found in the nests which were made of tubular plant materials which were open at both ends.

The artificial nesting sites were made of wooden blocks and tubular plant materials (reeds and bamboos) in the year 2019. The per cent occupancy in the artificial nests by arthropod nesters were not significant to each other except for the months *viz.,* June, July, November and December in the year 2019 (Table 29). Occupancy by ants and spiders was confined to the nests made of tubular plant materials whereas the occupancy by other arthropod nesters (*C. bengalense*, *Dasyproctus* sp. and *R. brunneum*) was confined to the nests made of wooden blocks. The per cent occupancy of nesting by solitary pollen bees towards the artificial nesting substrates was only significant in the month of May during the year 2019 (Table 30).

As the artificial nesting substrates with reeds and bamboos were not found much attractive towards the nesting of major solitary bee pollinators of cucurbit ecosystem, an extensive search for alternate nesting substrate was conducted in the year 2020 to provide more suitable substrate for domiciliation of native pollinating bees. Hollow dry twigs of solitary bee flora *viz*., *Caesalpinia pulcherrima*, *Lantana* spp., *Rosa* spp. and *Tecoma* sp. were thoroughly monitored for the presence of natural nests. The peacock flower tree, *C. pulcherrima* was found as the most preferred nesting host for the major solitary pollen bees of cucurbit ecosystem *viz*., *B. picitarsis*, *C. smaragdula* and *C. hieroglyphica* as the maximum number of active brood nests and full brood nests were discovered from the tree itself. Thus artificial nesting sites made of dried hollow twigs of *C. pulcherrima* were established during September 2020. The first occupancy of the solitary pollen bee, *C. smaragdula* was recorded within 62 days from the establishment of nest. The per cent nest occupancy of solitary

pollen bees towards the artificial nests made with *C. pulcherrima* twig material got increased from November 2020.

In the year 2020, the per cent occupancy of hymenopteran nesters other than the solitary pollen bee species was maximum in the wooden block nests, whereas ants and spiders were confined to the nests made with naturally available hollow twigs (Table 31 and Table 32). The per cent occupancy of solitary bees other than the megachilid bees was maximum in the nests made of naturally available hollow tubular plant materials. Megachilid bees showed species specific preference to nesting materials as *M. disjuncta* preferred wooden block nest with diameters of 7mm to 12 mm and *Megachile* sp. preferred nests made of locally available materials (reeds and bamboos) with a diameter of 6 mm to 9 mm.

In the year 2021, the observations on per cent occupancy of various arthropod species towards the nesting substrates established that hymenopteran nesters other than solitary bees preferred wooden blocks over naturally available tubular plant materials for domiciliation (Table 33 and Table 34). Solitary pollen bees other than megachilid bees showed specificity to natural host materials as nesting substrate. The solitary megachilid species were found to be nested in both wooden blocks as well as nests made of locally available plant materials.

4.3.3. Preference of distance towards the nesting of arthropod nesters

The preference of distance at which the artificial nests were placed from the cucurbitaceous ecosystem played a major role in nest occupancy by arthropod nesters.

In the year 2019, the arthropod species *viz*., *C. bengalense*, *Dasyproctus* sp. and *R. brunneum* were only found at nests which were placed 250 meters away from the field area. The ants and spiders were present at nests which were placed at distances of 10 m and 100 m away from the field, but they were not found at distance of 250 m away from the field. Solitary pollen bees were only found at nests which were placed at distance of 250 m away from the field. Similar observations were recorded in the years *i.e*., 2020 and 2021 (Table 29, 30, 31, 32, 33 & 34).

4.3.4. Combined effect of hole size and nesting substrates towards the nesting of arthropod nesters

The combined effect of hole size and nesting substrates towards arthropod nesters revealed significant impact on per cent nest occupancy during the years 2019, 2020 and 2021 (Table 29, 30, 31, 32, 33 and 34). Solitary pollen bees showed a preference for specific hole sizes for nest construction. Though megachilid bees nested in both nesting substrates, species specific preference towards nesting substrate was observed. Major solitary bee pollinators of cucurbit ecosystem *viz*., *B. picitarsis*, *C. smaragdula* and *C. hieroglyphica* were confined to nests made of naturally available host twigs with nest hole sizes *viz*., 2.5, 3 and 3.5 mm.

4.3.5. Combined effect of hole size and distance towards the nesting of arthropod nesters

The combined effect of hole size and distance towards the nesting of arthropod nesters showed significant impact on per cent occupancy in the years 2019, 2020 and 2021 (Table 29, 30, 31, 32, 33 and 34). Every species was specific to hole size diameters while construction of their nests. The arthropods other than ants and spiders showed preference to nests established at a distance of 250 m away from the main field area.

4.3.6. Combined effect of nesting substrates and distance towards the nesting of arthropod nesters

The combined effect of nesting substrates and distances towards the per cent occupancy of arthropod nesters varied at monthly intervals in all the three years for both solitary bee and non-solitary bee nesters. The effect of nesting substrates as well as distances to per cent occupancy of nesting by arthropod nesters was only significant in the months of January, February, May and June (Table 29) whereas the per cent occupancy of solitary pollen bee nesters was only significant in the month of May during the year 2019 (Table 30).

The combined effect of nesting substrates and distances from the field area to the per cent occupancy of arthropod nesters was not significant in the month of February, March, November and December (Table 31) whereas, it contributed

significantly to the per cent occupancy by solitary bees in the months of January, May, August, September and December (Table 32) during the year 2020.

The per cent occupancy by arthropod nesters was significantly influenced by the nesting substrates as well as the distances from the field area only during the months of February, April, May and July (Table 33) in the year 2021 whereas, it was significant in the nesting of solitary pollen bees in all the months except May and July (Table 34).

4.3.7. Combined effect of hole sizes, nesting substrates and distance towards the nesting of arthropod nesters

Different factors such as hole sizes, nesting substrates and distances from field area had a combined effect on the per cent nest occupancy of solitary and non-solitary nesters. The factors altogether contributed significantly to the nesting of arthropod nesters during the months of January, February, March, June, July and August (Table 29) during the year 2019 whereas, per cent occupancy by solitary bee nesters was only significant in the month of May (Table 30). Three factors contributed significantly to the per cent nest occupancy of arthopods in every months except in Februray, March and April (Table 31) in the year 2020, whereas it was significant only during the months of January, May, August, September and December for the per cent occupancy of solitary bee nesters (Table 32). The combined effect of all three factors contributed significantly towards the nesting of arthropods except for the month of May (Table 33) in the year 2021, whereas the effect of all three factors contributed significantly towards the per cent nest occupancy of solitary bee nesters in every month (Table 34).

4.3.8. Nesting architecture and nesting biology of two small carpenter bees

In the present study, the nesting architecture and nesting biology of small carpenter bees *viz., C. smaragdula* and *C. hieroglyphica* were studied as these were found to be the most promising solitary bee pollinators in cucurbit ecosystems. The small carpenter bees were found to nest in soft pithy and dry stems of *C. pulcherrima* trees linearly. A total of 199 nests were collected from *C. pulcherrima* trees which were planted at a distance of two meters. Out of 199 nests collected, 128 nests were inhabited by *C. smaragdula* and seventy one nests were inhabited by *C. hieroglyphica*. According to the classification of nests given by Daly (1966), nests

were classified and counted separately, where *C. smaragdula* nests comprised of 19 hibernacula, 28 founding nests, 21 active brood nests, 15 full brood nests and 45 mature brood nests. *C. hieroglyphica* comprised 8 hibernacula, 4 founding nests, 17 active brood nests, 11 full brood nests and 31 mature brood nests. The active and full brood nests of both the bee species were used to study the nest architecture (n=25, *C. smaragdula* and n=25, *C. hieroglyphica*). The nests of both species had only one entrance and the entrance diameter did not differ among *C. smaragdula* and *C. hieroglyphica* (two sample t-test, t=0.848, P>0.05) (Table 35). Most of the nests were found with adult bees guarding their nests either showing their head or abdomen to ward off natural enemies and thereby protecting their young ones. Preferences of bees towards twig thickness varied significantly ($t=^{-}3.365$, $P<0.05$) whereas, inner nest diameter showed only slight significant difference ($t=^{-}1.357$, $P>0.05$). Cells constructed inside were separated with pith of stem with a septum thickness of 3.1±0.10 and 2.70±0.08 in *C. smaragdula* and *C. hieroglyphica* respectively. Cells constructed in individual nests were equal to the length of adult bees and were arranged continuously with one after another without any empty space between them. Individual cell length of both the species ranged 6 to 10 mm with slight significant difference in length ($t=^{-}5.139$, $P<0.05$). Each cell was harboured with one immature stage except a few, and the older one among them was placed at the innermost side of nest whereas the younger one near to the entrance of nest. Both the species showed little significant difference in their nesting attributes *viz.*, occupied cell length ($t=^{-}2.651$, $P>0.05$), cell septum thickness (t=3.024, P>0.05), number of cells per nest ($t=^{-}1.568$, $P>0.05$) and number of immature stages per nests ($t=^{-}1.672$, $P>0.05$). Most of the nests collected were found with one or two adult bees guarding their nests, whereas some were absent with adult bees in them. Both the species constructed their nests at varied heights (*C. smaragdula*; 61.55±5.34 and *C. hieroglyphica*; 63.42±6.74, with no significant difference in their preference towards selection of nesting site from ground ($t=^{-}0.218$, $P>0.05$).

The females of *C. smaragdula* as well as *C. hieroglyphica* bees placed their pollen provisions which is a mixture of pollen grains and nectar in individual cells constructed in their nest. The pollen provisions were yellow to orange in colour (Plate 3A) which weighed 14.80±0.35 and 14.45±0.33 (Mean±SE in mg; n=15) in *C. smaragdula* and *C. hieroglyphica* respectively. The eggs were laid dorsally on

pollen provision to ensure immediate availability of food for the larvae. Eggs were translucent white in colour (Plate 3B) with cylindrical shape and convexed ends. Eggs hatched in 3 to 5 days in both the species of bees with no significant difference (Two sample t-test; t=2.861; P>0.05) (Table 36). The first instar apodous larvae (Plate 3C) were translucent white in colour which actively fed on pollen provisions. Size of pollen mass varied in each cell of an active brood nest based on the stage of immatures present in them. Pollen masses were larger in size with early instars of larvae and vice-versa in cells with mature larvae. The first instar larvae were recognised as one by third size of pollen mass and which showed slight significant difference in their developmental days in *C. smaragdula* and *C. hieroglyphica* ($t=^{-}0.690$, P<0.05). The larva with two by third size of pollen mass (Plate 3D) in both the species of bees showed significant difference in their development period (t=0.695, P<0.05), whereas larva with twice the size of pollen mass did not show significant difference in their development period (t=0.402, P>0.05). Post defecating larvae were always found in their cells with feces and were metamorphosed into white coloured pupa. Pupa appeared with a difference in eye colour *viz*., white (Plate 3E), pale pink, pink (Plate 3F), pale brown, brown (Plate 3G) and black (Plate 3H) in accordance to the development period. Pupa with black coloured eye showed a difference in body pigmentation at different stages. Total pupal period of *C. smaragdula* ranged from 20.71±0.26 days whereas *C. hieroglyphica* ranged from 18.56±0.16 days. Adult longevity was also studied at laboratory conditions with 10 per cent honey solution. *C. smaragdula* showed an adult longevity of 8.55±0.36 days, whereas *C. hieroglyphica* showed 4.82±0.31 days which were not significantly different (t=7.706, P>0.05) among both species. The total developmental period of both the bee species could not be ascertained in the present study as the adult longevity period might vary based on climate, host plants and various other factors.

Table 35. Nesting architecture of two small carpenter bees

Particulars	*Ceratina smaragdula* nest	*Ceratina hieroglyphica* nest
Entrance diameter (mm)	2.92±0.07	3±0.05
Twig thickness (mm)	8.08±0.22	9.44±0.33
Nest thickness (mm)	3.14±0.06	3.33±0.09
Occupied nest length (cm)	7.03±0.47	8.82±0.48
Cell septum thickness (mm)	3.1±0.10	2.70±0.08
Individual cell length (mm)	6.08±0.53	4.32±0.76
Number of cells/nest	4.92±0.25	5.56±0.31
Number of immatures/nest	4.68±0.26	5.36±0.31
Number of adult/nest	1.00±0.05	0.80±0.08
Height of nest from ground level (cm)	61.55±5.34	63.42±6.74

Mean±SE, n=25

Table 36. Nesting biology of two small carpenter bees

Life stage description	*Ceratina smaragdula*	*Ceratina hieroglyphica*
Egg	4.36±0.12	3.87±0.11
Larva		
One third of PB	2.82±0.09	2.91±0.07
Two third of PB	2.74±0.08	2.62±0.14
Twice the size of PB	2.88±0.08	2.83±0.08
Pre-defecating larva	3.34±0.06	3.38±0.09
Post- defecating larva	3.71±0.10	4.17±0.15
Total larval period	15.51±0.19	15.93±.27
Pupa		
White eyed pupa	2.93±0.09	2.82±0.10
Pale pink eyed pupa	1.32±0.10	1.37±0.07
Pink eyed pupa	1.09±0.03	1.12±0.04
Pale brown eyed pupa	1.61±0.10	1.16±0.04
Brown eyed pupa	1.36±0.09	1.71±0.08
Black eyed pupa	2.81±0.09	3.02±0.07
½ body pigmented pupa	3.01±0.06	2.17±0.04
¾ body pigmented	2.53±0.14	1.53±0.09
Full body pigmented	4.01±0.08	3.62±0.10
Total pupal period	20.71±0.26	18.56±0.16
Adult		
Adult longevity	8.55±0.36	4.82±0.31
Total life cycle	49.15±0.40	43.18±0.58

Mean±SE, n=30

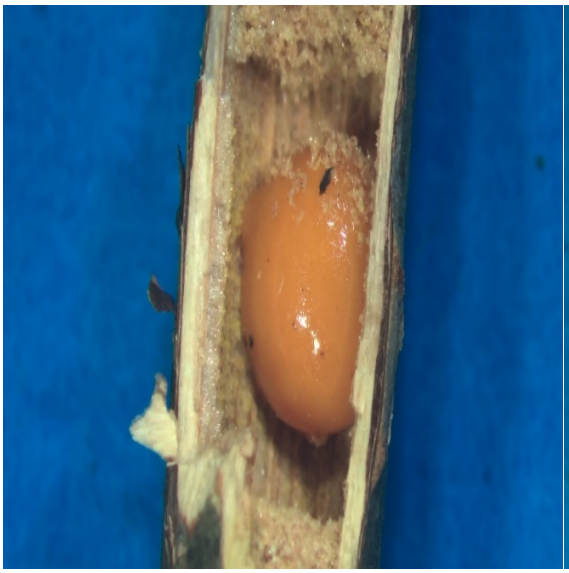


Plate 3A. Pollen provisions at nests of *Ceratina* bees

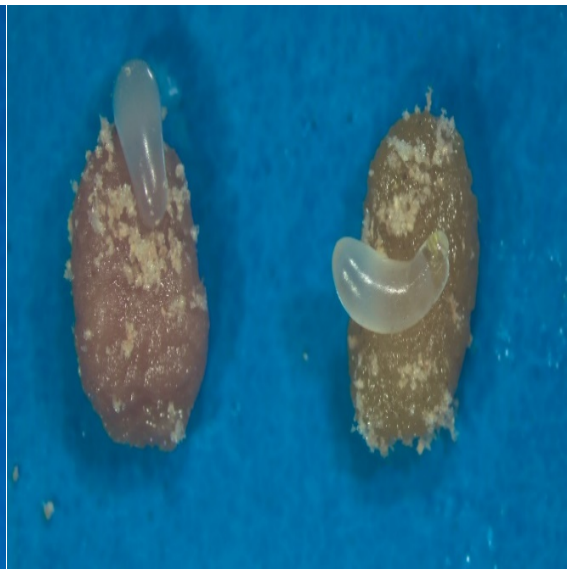


Plate 3B: Eggs of *Ceratina* bees

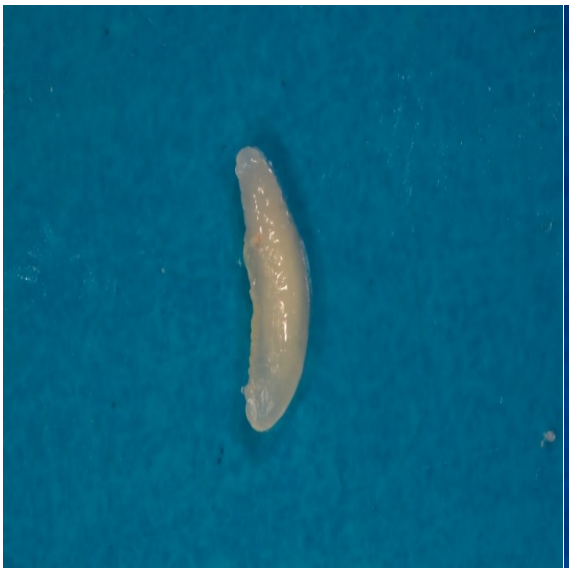


Plate 3C. First instar larva

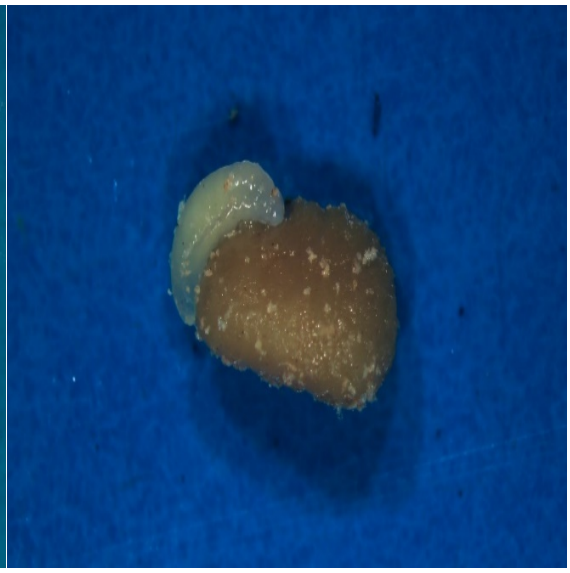


Plate 3D: Larva with two third size of pollen mass

Plate 3: Nesting biology of *Braunsapis picitarsis* (Cameron)

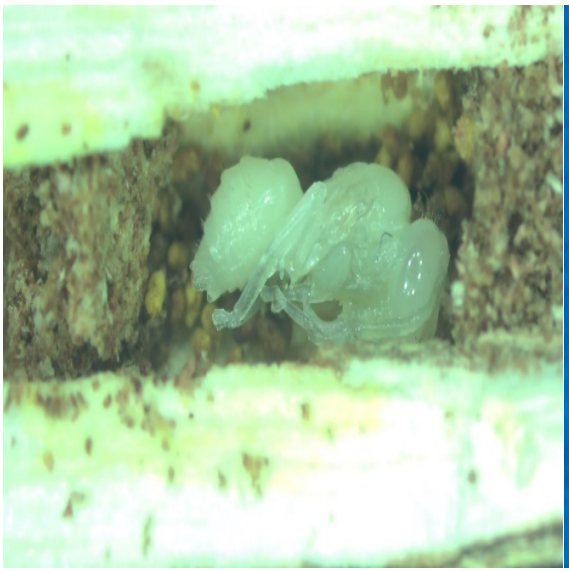


Plate 3E: White eyed pupa

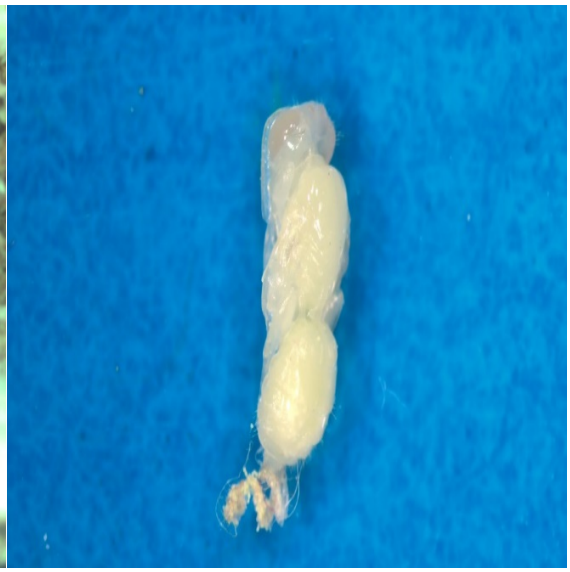


Plate 3F: Pink eyed pupa

Plate 3G: Dark brown eyed pupa

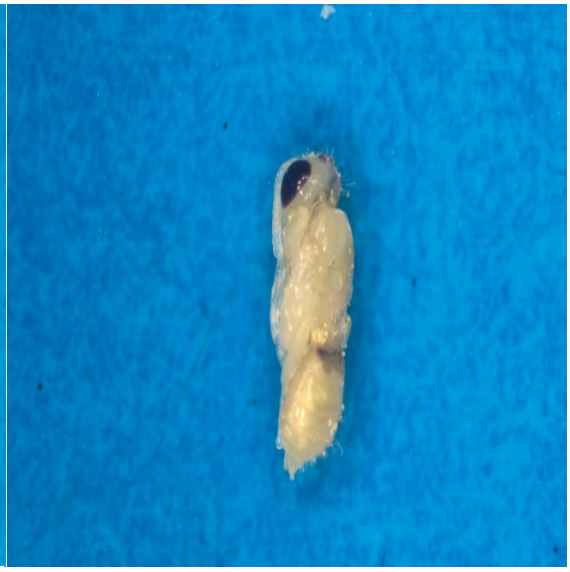


Plate 3H: Black eyed pupa

Plate 3: Nesting biology of *Braunsapis picitarsis* (Cameron)

4.3.8. Nesting architecture and nesting biology of allodapine bee, *Braunsapis picitarsis* (Cameron)

Braunsapis picitarsis is a major native bee pollinator of cucurbit crops and the intensive searches for locating their habitat revealed the presence of their nesting sites in dried and pruned stems of peacock flower tree, *Caesalpinia pulcherrima*. Apart from that, a limited number of nests were located in plants like *Rosa* spp. and *Lantana camara*. As the number of nests in those was limited, the studies on nest architecture and life cycle of *B. picitarsis* were done using the nesting sites in *C. pulcherrima* itself.

B. picitarsis constructed linear nests in soft pithy stems of *C. pulcherrima*, which were unbranched with an average length of 5.38±0.38 cm (N=30) and a twig thickness of 6.96±0.12 (Table 37). Out of the 83 nests collected, 7.22 per cent were hibernacula nest in which most of them were abandoned and some of them were occupied by adult bees. Active brood nests were about 9.63 per cent followed by founding nests (13.25 %) and full brood nests (69.87 %).

Female bee was found in a defensive position either showing their head or abdomen facing upward to guard their immatures inside the nest. The entrance diameter of the nests in *B. picitarsis* was found to be 2.83±0.06 (mm) and the nests were averaged with an internal thickness of 2.90±0.03 cm, in which pollen was spread throughout the extreme end of the nest wall. Eggs were laid in groups towards the innermost end of the nest where all of them were coated with pollen grains, ensuring immediate availability of food for the hatching larvae. There were no separate pollen provisions for the immature stages of *B. picitarsis* (plate 4A) compared to the common stem nesting small carpenter bees (*Ceratina* spp.) in which each eggs in the nest were deposited in separate pollen provisions procured by the mother bees. All the immatures were arranged linearly in the nest (Plate 4B) with matured ones placed towards the outermost end, whereas the younger ones were placed towards the innermost end of the nest. Mother bees continuously interacted with their offsprings as there were no cell partitioning and this might have helped the mother bees to properly provision and monitor their broods. The immatures averaged in 3 to 11 numbers in nests with one or two adult bees guarding them. There was a huge variation in height of the nests from ground level where it averaged 100.58±10.25 cm.

The life cycle of *B. picitarsis* consisted of egg, larva, pupa and adult with a total development period of 56.85±0.84 days (Table 38). The adult bees laid eggs in groups to the innermost end of the nests and hatched with an average of 4.44±0.14 days in the laboratory conditions. The eggs were banana shaped and translucent white which were mostly laid in groups (Plate 4C), and were covered with pollen grains from the nest wall. The apodous larva fed actively on the pollen grains and it took an average of 14.62±0.25 days to complete their larval period (Plate 4D). The post-defecated larva metamorphosed into white eyed pupae (Plate 4E) which showed various eye pigmentation from pink, brown and black (Plate 4F). The black eyed pupa gradually attained pigmentation in the body and the total pupal period lasted an average of 21.11±0.32 days. The adult longevity was only up to 16.66±0.64 days under laboratory conditions.

Table 37. Nesting architecture of allodapine bee, *Braunsapis picitarsis*

Particulars	(Mean±SE) (N=30)	Range
Entrance diameter (mm)	2.83±0.06	2.10-3.50
Twig thickness (mm)	6.96±0.12	5.50-8.00
Nest thickness (mm)	2.90±0.03	2.50-3.00
Occupied nest length (cm)	5.38±0.30	3.00-9.00
Number of immatures/nest	7.24±0.31	3.00-13.00
Number of adult/nest	0.96±0.09	0.00-2.00
Height of nest from ground level (cm)	100.58±10.25	17.90-172.40

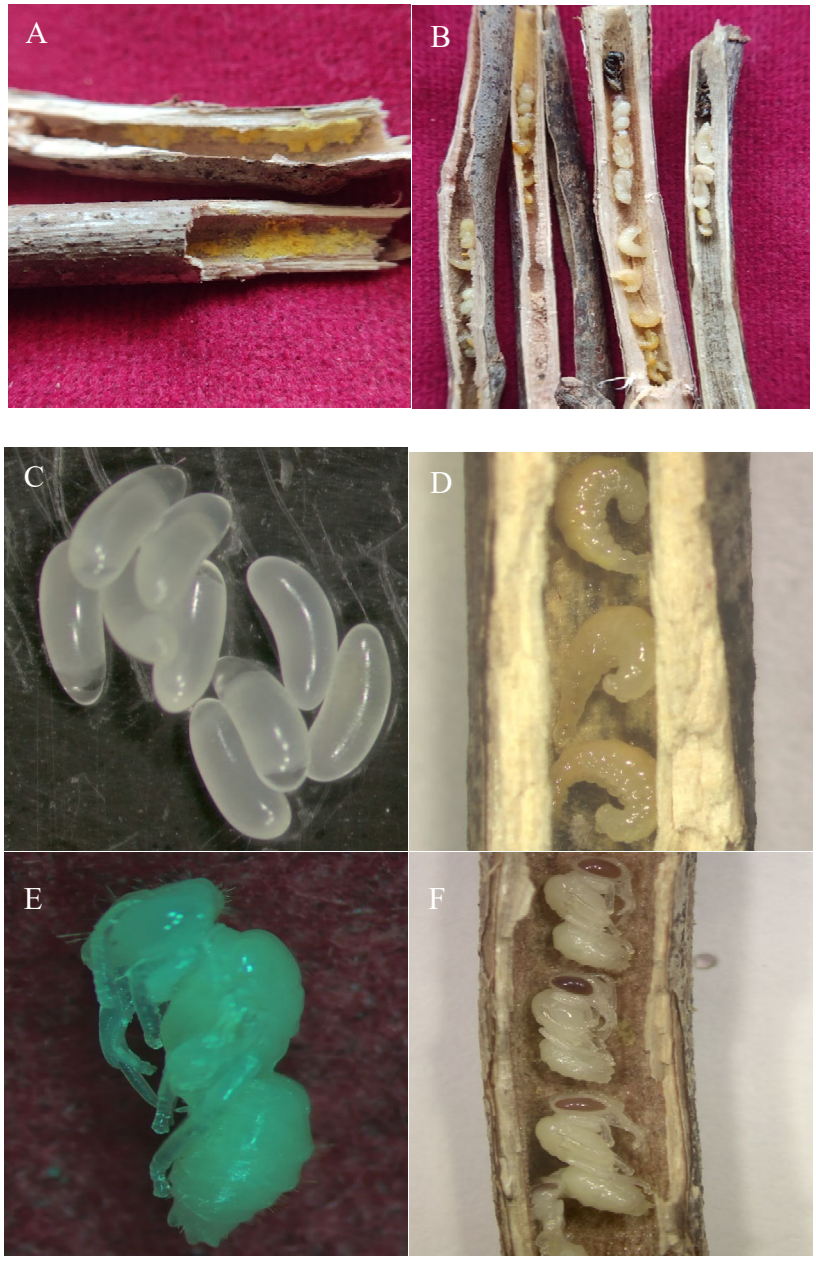


Plate 4: A - pollen provisions in *Braunsapis* nest, B- linearly arranged immature stages of *Braunsapis* bees inside *Caesalpinia* twigs, C- *B. picitarsis* eggs laid in group D - *B. picitarsis* larva in bee nest, E- white eyed pupa of *B. picictarsis*, F- pink, brown and black eyed pupa of *B. picitarsis* inside bee nest

Table 38. Nesting biology of allodapine bee, *Braunsapis picitarsis*

Life stage description	*Braunsapis picitarsis* (N=30)	Range
Egg	4.44±0.14	3.18-7.00
Larva		
First instar larva	3.17±0.11	2.12-4.12
Second instar larva	2.66±0.08	2.00-3.60
Third instar larva	2.91±0.07	2.00-4.00
Pre-defecating larva	3.39±0.09	3.00-4.60
Post-defecating larva	2.48±0.09	2.00-3.60
Total larval period	14.62±0.25	12.18-17.12
Pupa		
White eyed pupa	3.57±0.09	3.00-4.60
Pink eyed pupa	1.51±0.07	1.00-2.00
Brown eyed pupa	2.60±0.10	1.18-3.60
Black eyed pupa	3.20±0.13	2.12-4.60
Pupa with 1/2 body pigmentation	2.86±0.09	2.00-3.60
Pupa with 3/4 body pigmentation	2.73±0.09	2.00-3.60
Pupa with full body pigmentation	4.62±0.12	3.18-5.60
Total pupal period	21.11±0.32	17.60-24.22
Adult longevity	16.66±0.64	11.12-24.60
Total life cycle	56.85±0.84	49.32-66.46

4.4 Study on palynology of solitary pollen bees

Palynological studies were conducted to observe the diversity of pollen collected by solitary pollen bees. The pollen grains collected from either pollinator's body or their natural and artificial nesting sites were subjected to light microscopy as well as scanning electron microscopy to observe the pollen diversity. The majority of pollen collected from the bee body surface and their nesting sites belonged to the family Fabaceae (27 %) followed by Asteraceae (11 %), Cucurbitaceae (11 %), Lamiaceae (11 %), Portulacaceae (5 %), Passifloraceae (5 %), Rubiaceae (5 %), Acanthaceae (5 %), Oxalidaceae (5 %) and, Polygonaceae (5 %).

A total of 19 pollen grains were identified in the present study, which varied in their shape, size, aperture type and exine pattern (Table 39) (Plate 5A - 5s). Pollen grains recorded under the family Fabaceae were spheroidal, circular, or triangular with their pollen dispersal unit as monad or tetrad. The size of pollen grains varied greatly from small, medium to large with most of the pollen grain having tricolporate or tricolpate aperture with various exine patterns. Major flora recorded were peacock flower tree (*Caesalpinia pulcherrima*), butterfly pea (*Clitoria* sp.), Indian hemp (*Crotaleria* sp.), touch-me-not-plant (*Mimosa pudica*) and snowy orchid tree (*Bauhinia acuminata*).

Pollen grains recorded under the family Cucurbitaceae were prolate, triangular and spheroidal in shape with pollen dispersal unit as monad. Most of the pollen grains were large in size having tricolporate or porate apertures and reticulate or verrucate exine patterns. Major flora recorded under the family were, bitter gourd (*Momordica charantia*), oriental pickling melon (*Cucumis melo* var. *conomon*) and, snake gourd (*Trichosanthes anguina*).

Pollen grains recorded under the family Asteraceae were spheroidal in shape having monad pollen dispersal units. The pollen grains were medium sized having tricolporate aperture and echinate exine patterns. Major flora recorded under the family were Singapore daisy (*Sphagneticola trilobata*), common zinnia (*Zinnia* sp.) and tridax daisy (*Tridax procumbens*).

Pollen grains recorded under the family Lamiaceae were either spheroidal or triangular with monad dispersal units. They were medium sized with hexacolpate or

Plate 5A) Light microscopic image of *Caesalpinia pulcherrima*

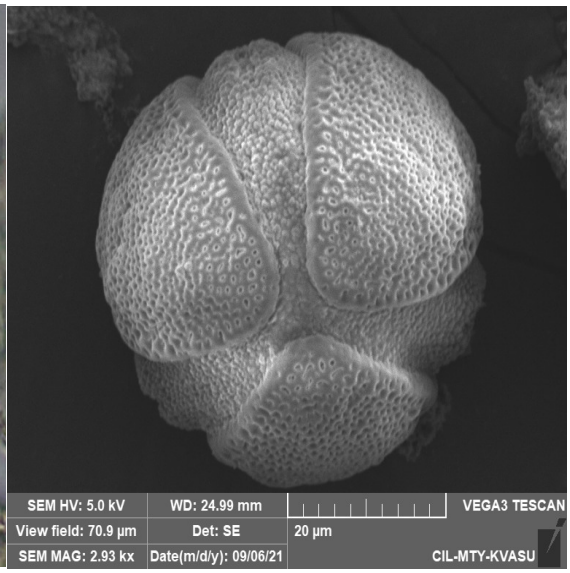


Plate 5a) SEM image of *Caesalpinia pulcherrima*

Plate 5B) Light microscopic image of *Clitoria* sp.

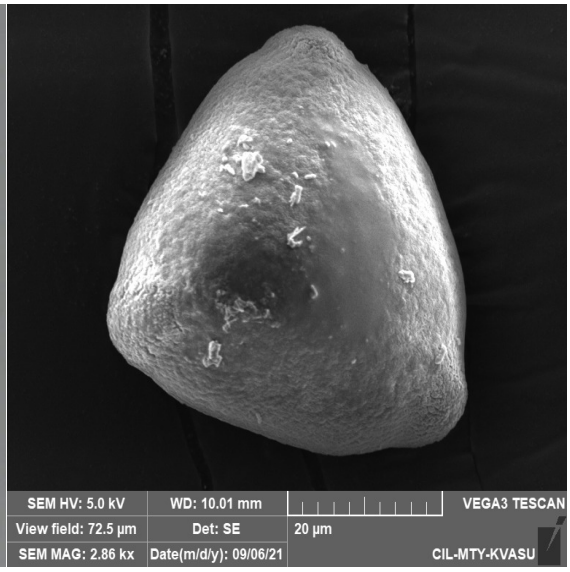


Plate 5b) SEM image of *Clitoria* sp.

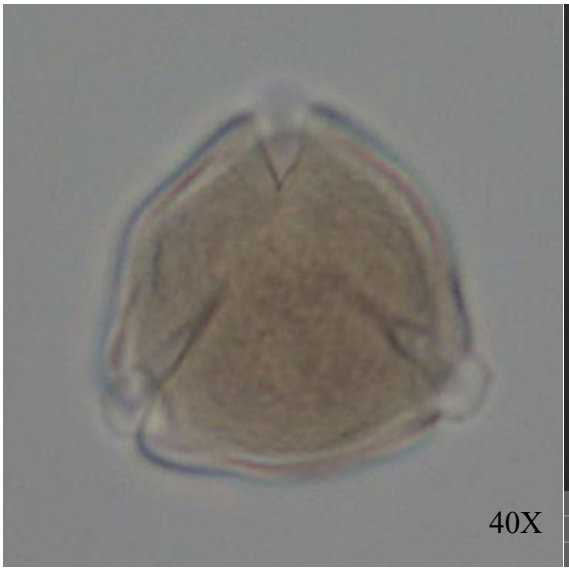


Plate 5C) Light microscopic image of *Crotaleria* sp.

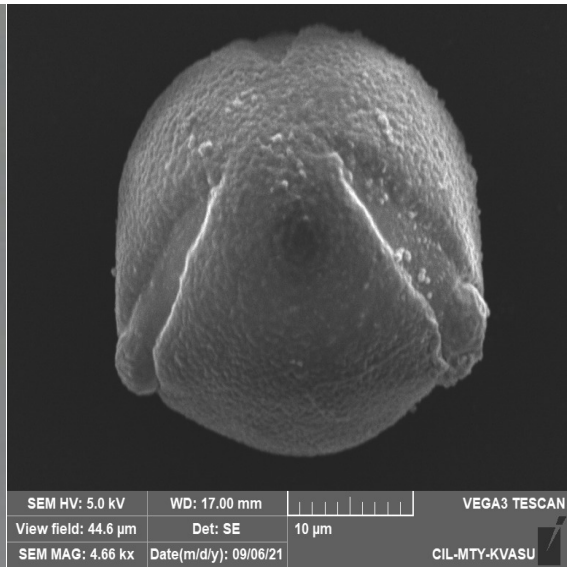


Plate 5c) SEM image of *Crotaleria* sp.

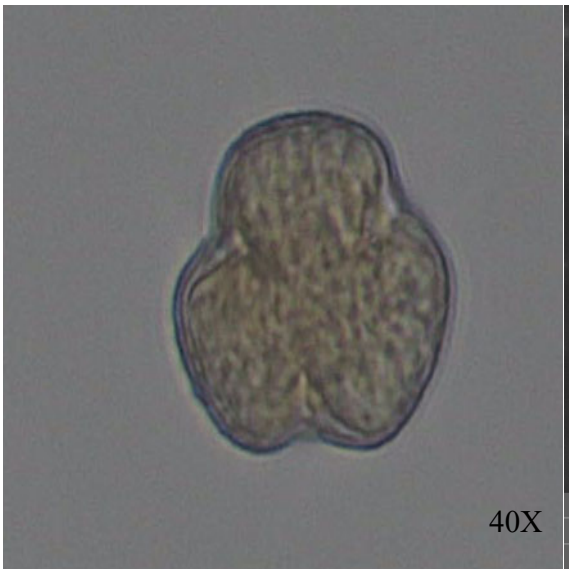


Plate 5D) Light microscopic image of *Mimosa pudica*

Plate 5d) SEM image of

Mimosa pudica

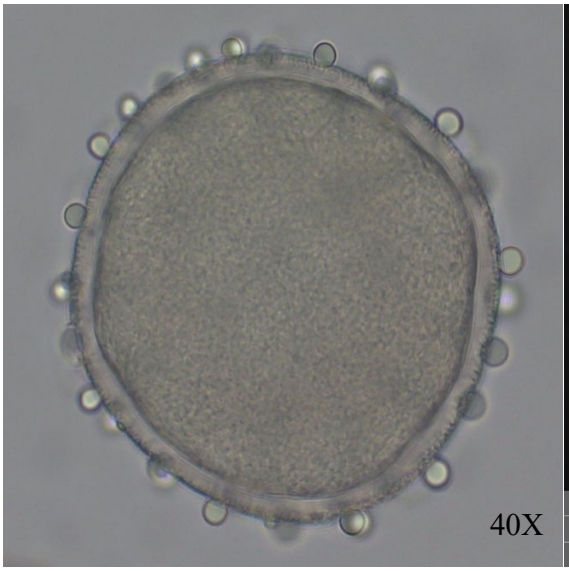


Plate 5E) Light microscopic image of *Bauhinia* sp.

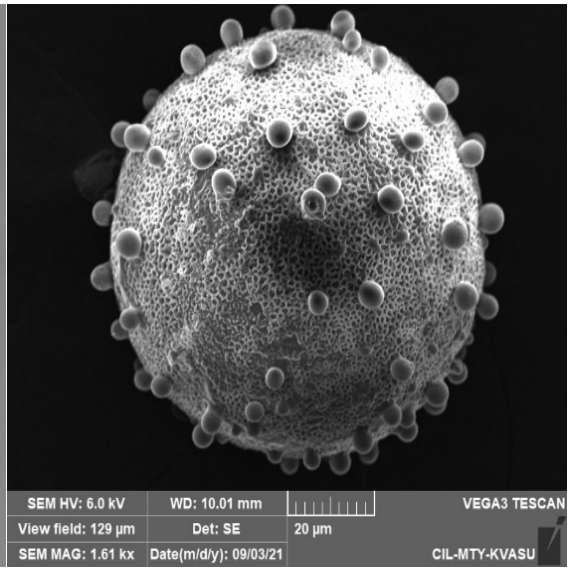


Plate 5e) SEM image of *Bauhinia* sp.

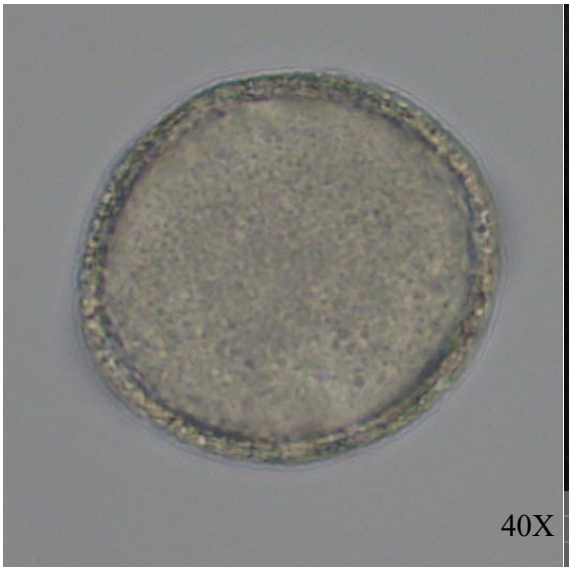


Plate 5F) Light microscopic image of *Momordica charantia*

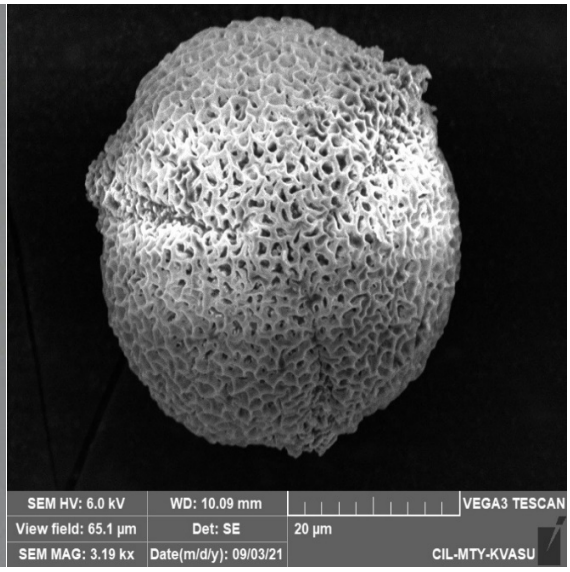
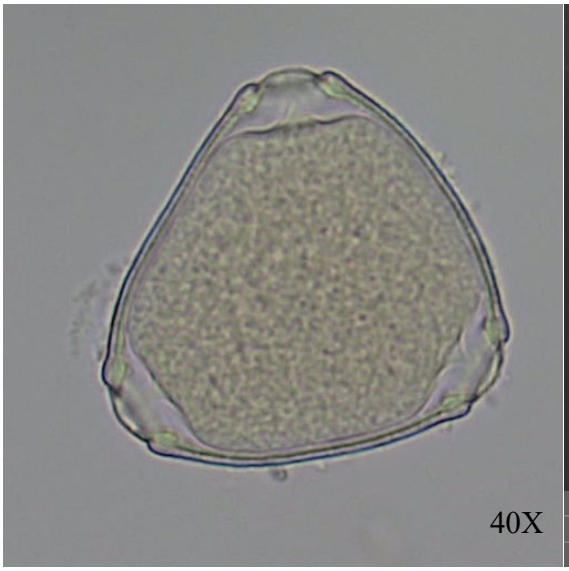


Plate 5f) SEM image of *Momordica charantia*



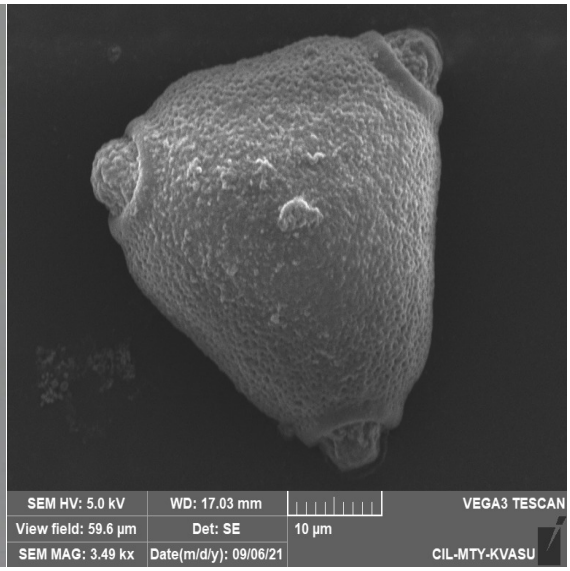
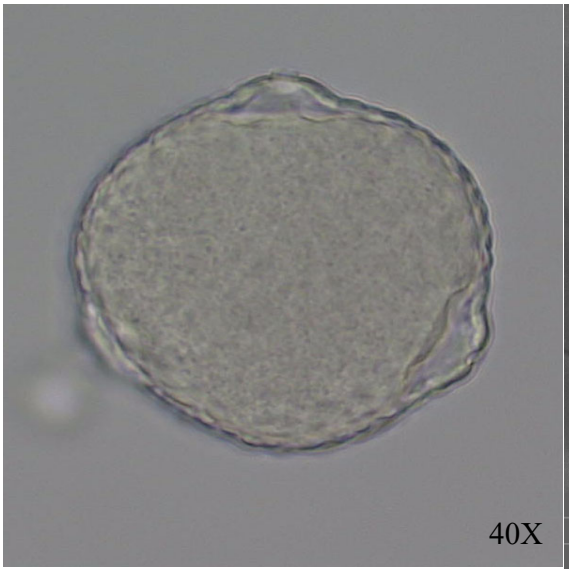


Plate 5G) Light microscopic image of *Cucumis melo* var. *conomon*

Plate 5g) SEM image of *Cucumis melo* var. *conomon*



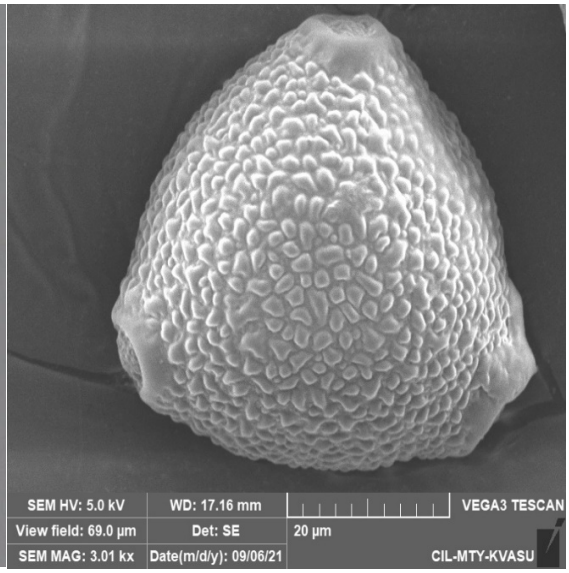
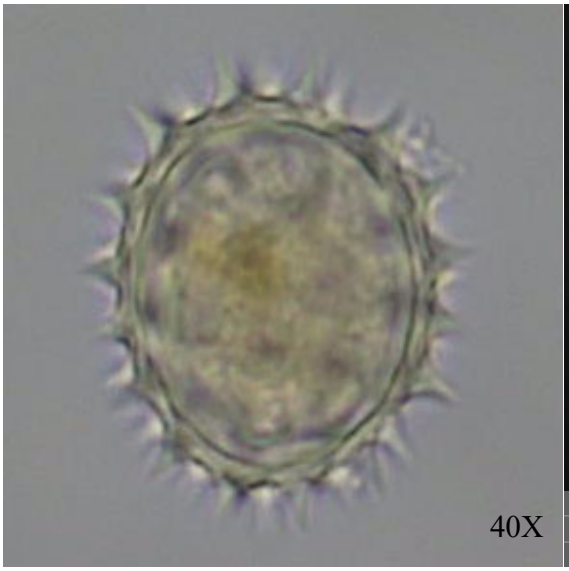


Plate 5H) Light microscopic image of *Trichosanthes anguina*

Plate 5h) SEM image of *Trichosanthes anguina*



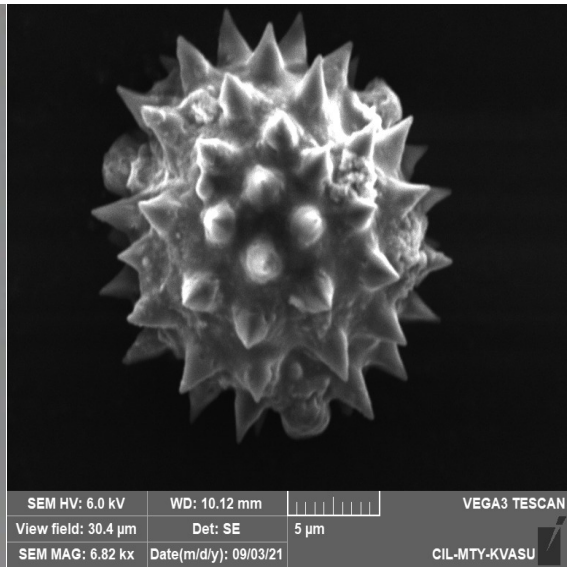
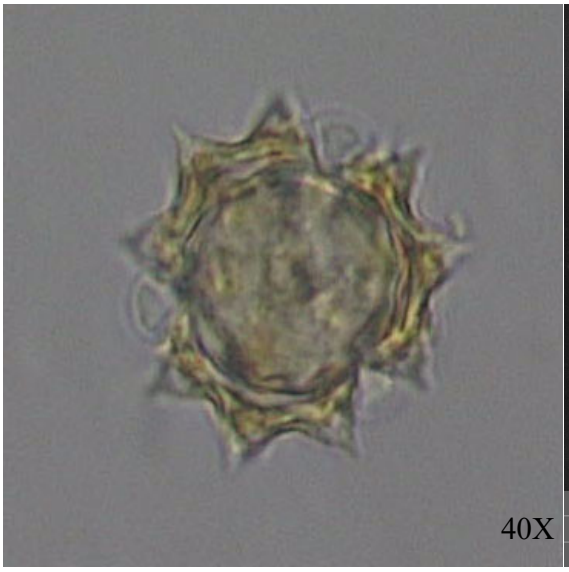


Plate 5I) Light microscopic image of *Sphagneticola trilobata*

Plate 5i) SEM image of *Sphagneticola trilobata*



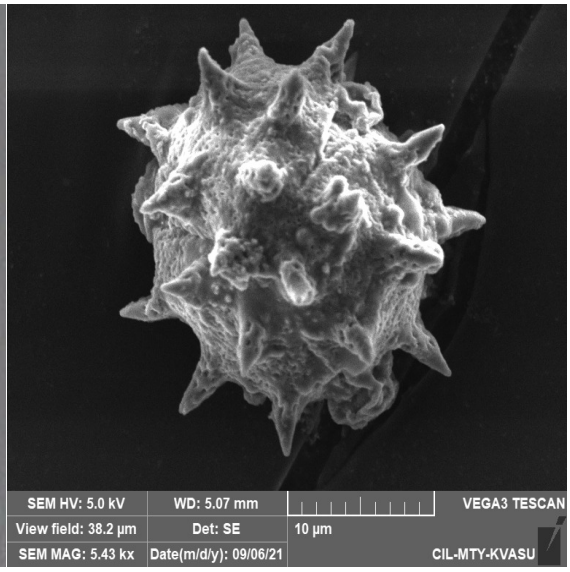


Plate 5J) Light microscopic image of *Zinnia* sp.

Plate 5j) SEM image of *Zinnia* sp.

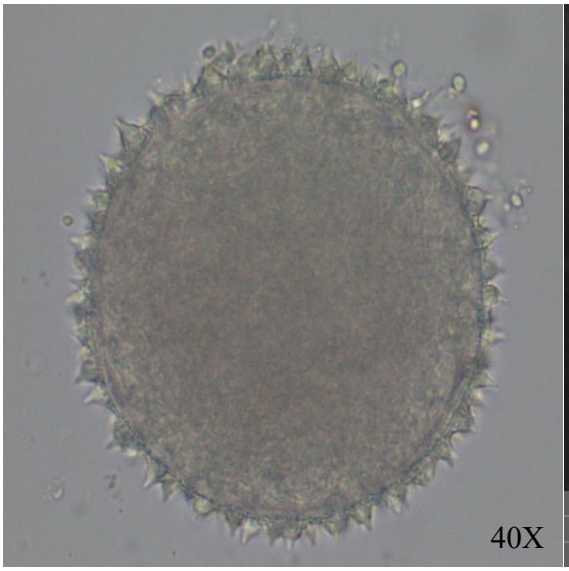


Plate 5K) Light microscopic image of *Tridax procumbens*

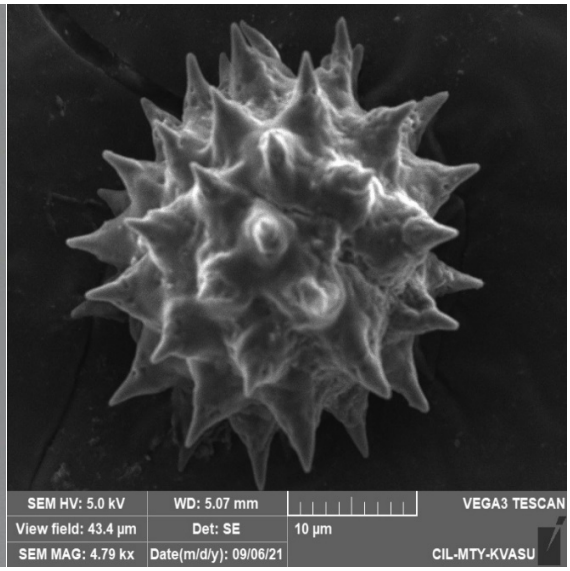


Plate 5k) SEM image of *Tridax procumbens*

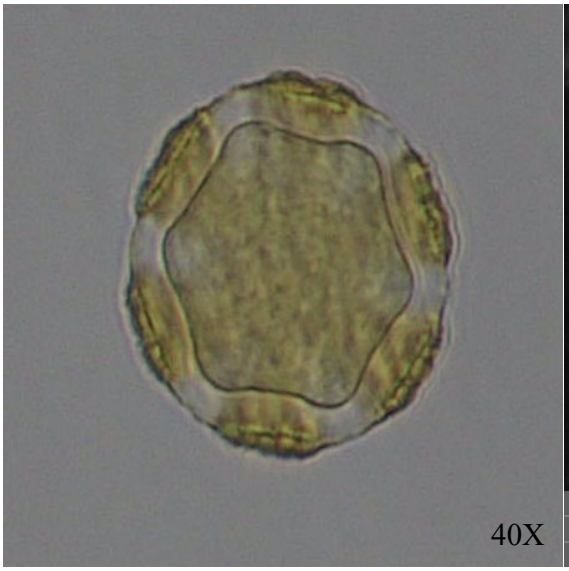


Plate 5L) Light microscopic image of *Ocimum tenuiflorum*

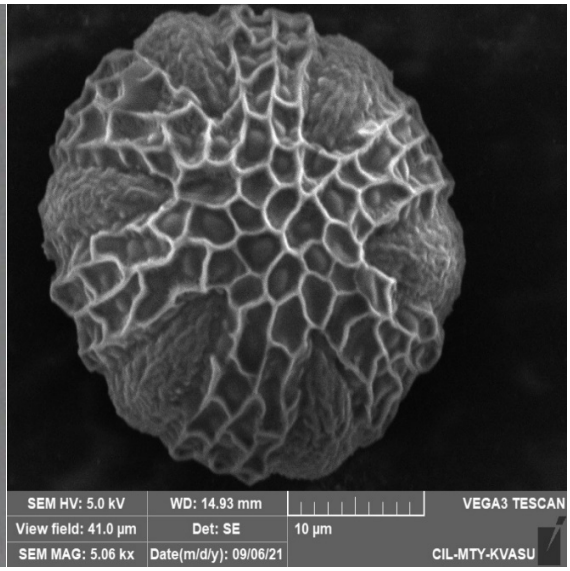


Plate 5l) SEM image of *Ocimum tenuiflorum*

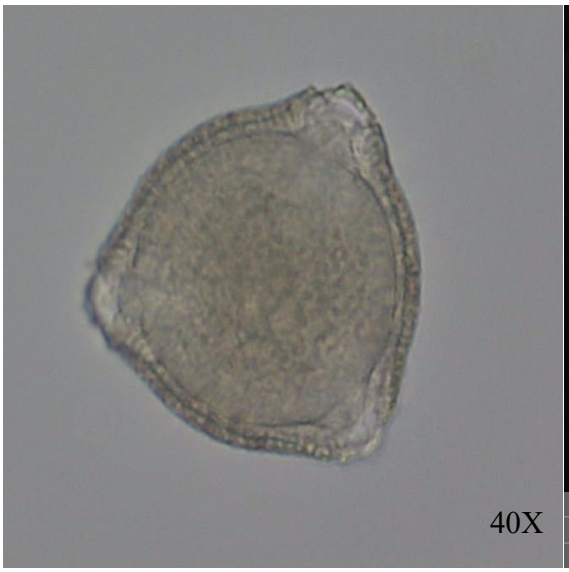


Plate 5M) Light microscopic image of *Pogostemon* sp.

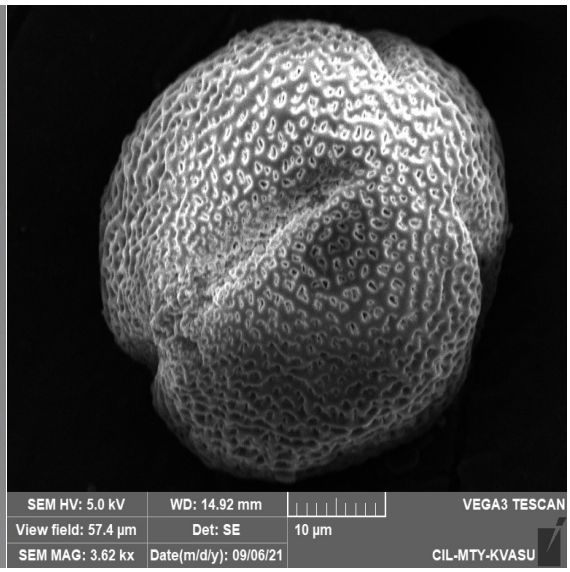


Plate 5m) SEM image of *Pogostemon* sp.

Plate 5N) Light microscopic image of *Thunbergia grandiflora*

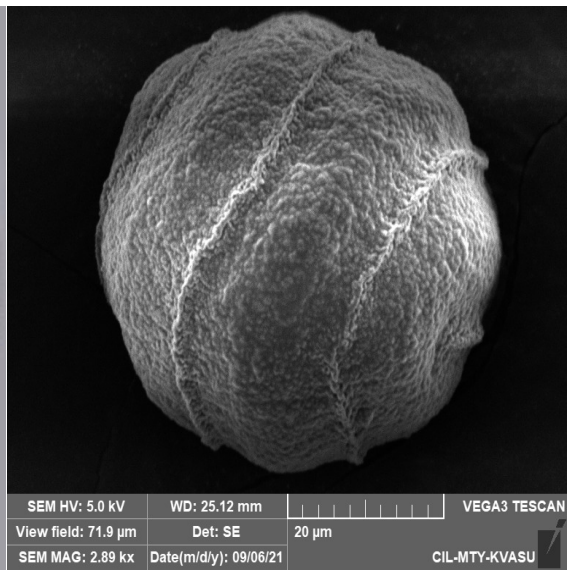


Plate 5n) SEM image of *Thunbergia grandiflora*

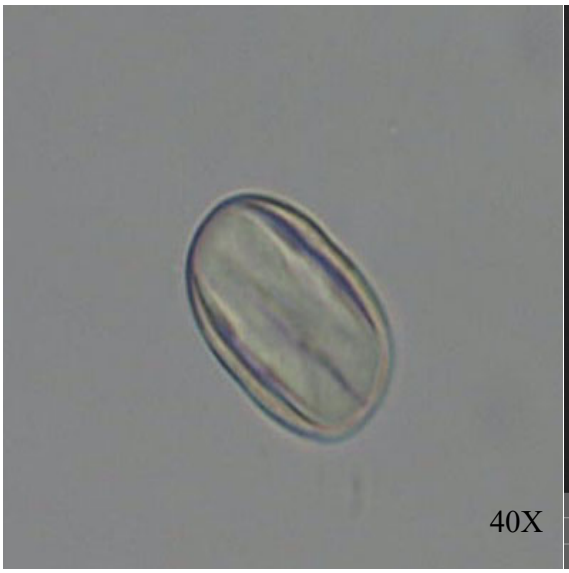


Plate 5O) Light microscopic image of *Biophytum sensitivum*

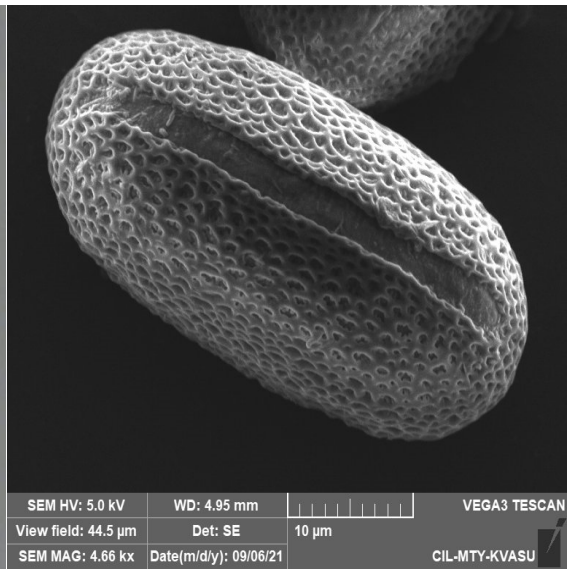


Plate 5o) SEM image of *Biophytum sensitivum*

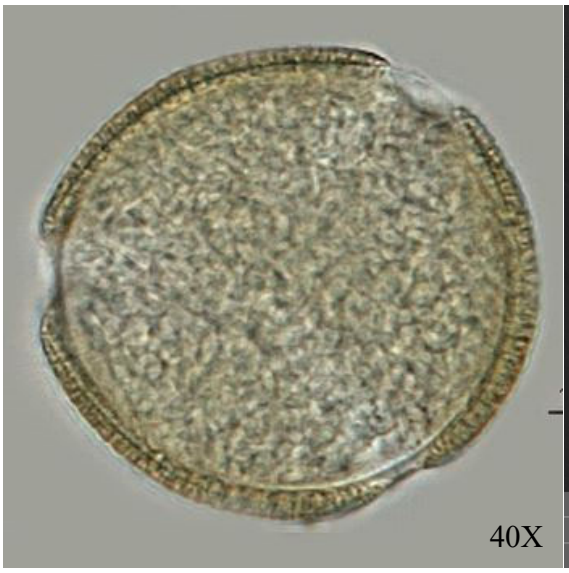


Plate 5P) Light microscopic image of *Antigonon leptopus*

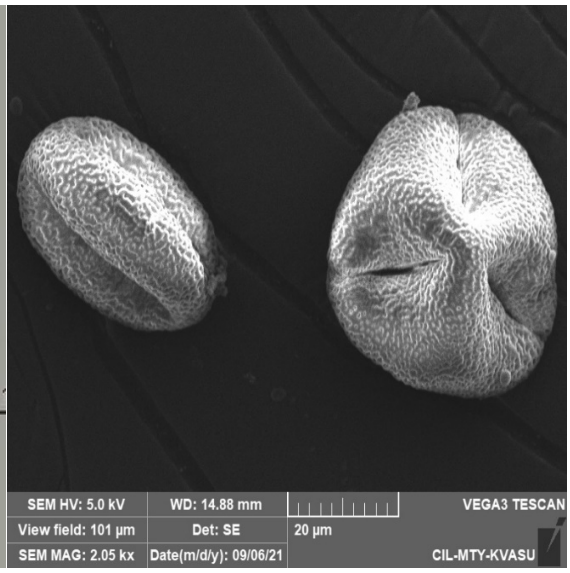


Plate 5p) SEM image of *Antigonon leptopus*

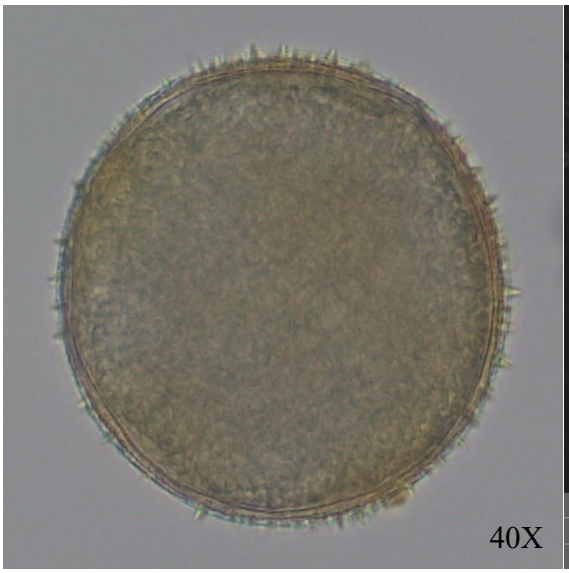


Plate 5Q) Light microscopic image of *Portulaca grandiflora*

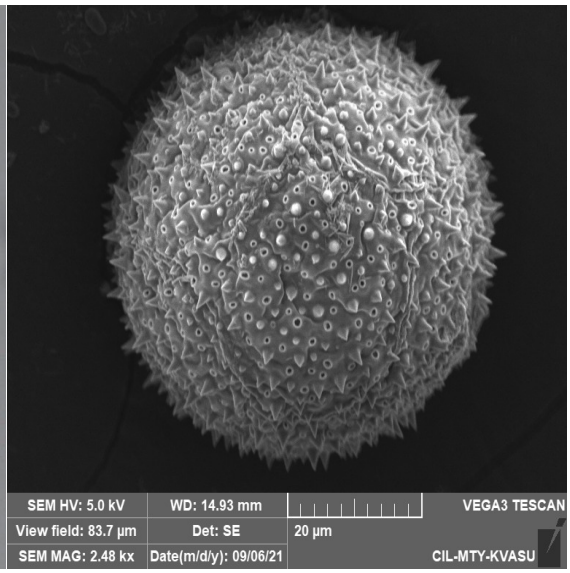


Plate 5q) SEM image of *Portulaca grandiflora*

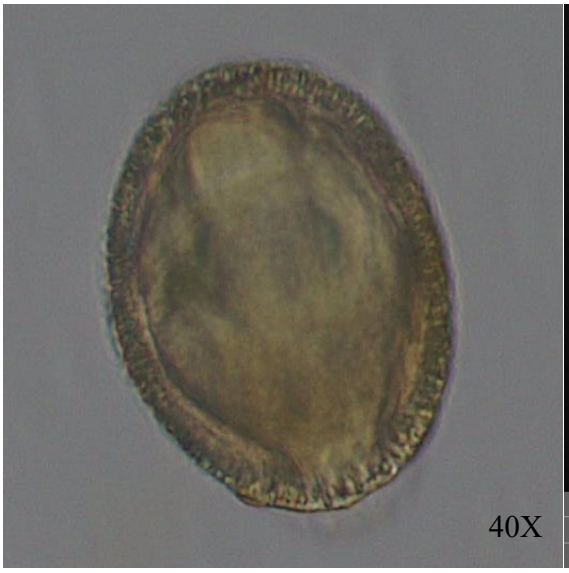


Plate 5R) Light microscopic image of *Turnera* sp.

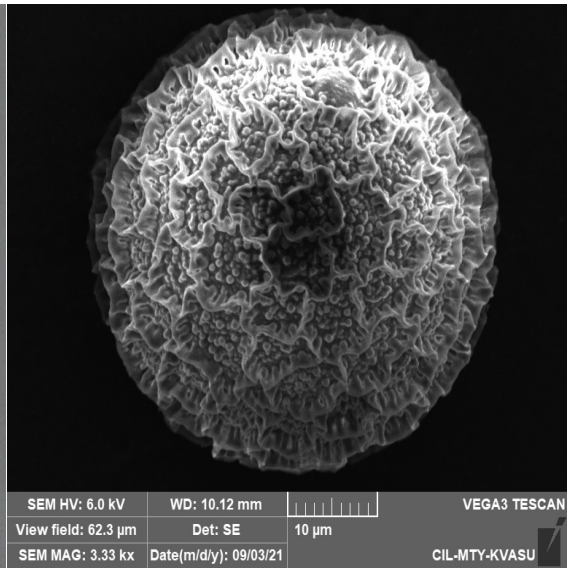


Plate 5r) SEM image of *Turnera* sp.

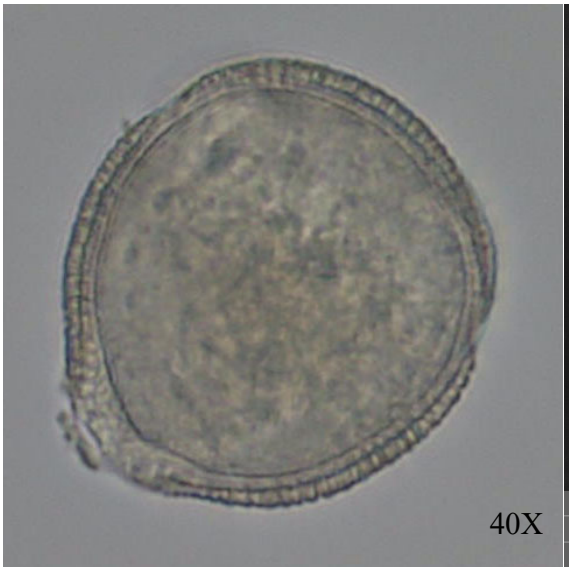


Plate 5S) Light microscopic image of *Hamelia patens*

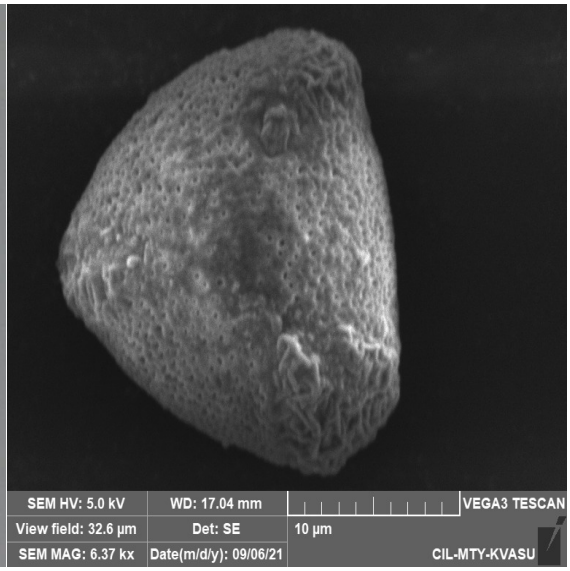


Plate 5s) SEM image of *Hamelia patens*

tricolporate aperture and reticulate or perforate exine pattern. Major flora recorded under this family were basil (*Ocimum* sp.) and Bengal shrub-mint (*Pogostemon* sp.).

The pollen grain recorded under the family Acanthaceae was of the Bengal clock vine (*Thunbergia grandiflora*) flower which had a circular shape and monad pollen dispersal unit. The pollen grain was large having spiraperturate aperture and verrucate exine pattern. The pollen grain recorded under the family Oxalidaceae was of the little tree plant (*Biophytum sensitivum*) with spheroidal shape and monad pollen dispersal units. The pollen grain was medium sized having tricolpate aperture and reticulate exine pattern. The pollen grain recorded under the family Polygonaceae was of the coral vine flower (*Antigonon leptopus*) having spheroidal shape and monad pollen dispersal units. The pollen grain was large sized having tricolporate aperture and reticulate exine pattern. The pollen grain recorded from the family Portulacaceae was of the moss rose purslane flower (*Portulaca grandiflora*) with a circular shape and monad pollen dispersal units. It was large having pantocolpate aperture and echinate exine pattern. The pollen grain recorded from the family Passifloraceae was of the white buttercup flower (*Turnera* sp.) with oblate shape and monad pollen dispersal units. The pollen was large with a tricolporate aperture and reticulate exine pattern. The pollen grain recorded from the family Rubiaceae was of the scarlet bush flower (*Hamelia patens*) with spheroidal shape and monad pollen dispersal units. The pollen was medium sized with tricolpate aperture and foveolate exine pattern.

It was found that most abundant pollen grains in the nest of the small carpenter bees, *C. hieroglyphica* and *C. smaragdula* and the allodapine bee, *B. picitarsis* were of the peacock flower trees. The most abundant pollen grains found in the nest of the megachilid bee, *M. disjuncta* was from Singapore daisy flowers. All the other pollen grains found in the nesting sites were very less in number. Pollen grains collected from the body surface of foraging solitary bees varied in their number greatly.

Table 39. Pollen morphological descriptions associated with bee flora

Sl. No.	Taxa	Pollen grain shape	Dispersal unit	Size	Aperture	Exine pattern
Fabaceae						
1	*Caesalpinia pulcherrima*	Spheroidal	Monad	49.76 µm	Tricolporate	Reticulate
2	*Clitoria* sp.	Triangular	Monad	49.54 µm	Tricolpate	Microrugulate
3	*Crotaleria* sp.	Circular	Monad	29.95 µm	Tricolpate	Verrucate
4	*Mimosa pudica*	Spheroidal	Tetrad	7.04 µm	Inaperturate	Psilate
5	*Bauhinia acuminata*	Spheroidal	Monad	42.86 µm	Tricolporate	Striate
Cucurbitaceae						
6	*Momordica charantia*	Prolate	Monad	52.35 µm	Tricolporate	Reticulate
7	*Cucumis melo* var. *conomon*	Triangular	Monad	42.21 µm	Triporate/Porate	Reticulate
8	*Trichosanthes anguina*	Spheroidal	Monad	52.43 µm	Porate	Verrucate
Asteraceae						
9	*Sphagneticola trilobata*	Spheroidal	Monad	21.40 µm	Tricolporate	Echinate
10	*Zinnia* sp.	Spheroidal	Monad	23.97 µm	Tricolporate	Echinate
11	*Tridax procumbens*	Spheroidal	Monad	25.90 µm	Tricolporate	Echinate
Lamiaceae						
12	*Ocimum* sp.	Spheroidal	Monad	32.24 µm	Hexacolpate	Reticulate

13	*Pogostemon* sp.	Triangular	Monad	26.96 µm	Tricolporate	Perforate
Acanthaceae						
14	*Thunbergia grandiflora*	Circular	Monad	52.35 µm	Spiraperturate	Verrucate
Oxalidaceae						
15	*Biophytum sensitivum*	Spheroidal	Monad	28.44 µm	Tricolpate	Reticulate
Polygonaceae						
16	*Antigonon leptopus*	Spheroidal	Monad	42.14 µm	Tricolporate	Reticulate
Portulacaceae						
17	*Portulaca grandiflora*	Circular	Monad	57.19 µm	Pantocolpate	Echinate
Passifloraceae						
18	*Turnera* sp.	Oblate	Monad	44.90 µm	Tricolporate	Reticulate
Rubiaceae						
19	*Hamelia patens*	Spheroidal	Monad	21.68 µm	Tricolpate	Foveolate

4.5 Determination of effect of different plant protection measures on pollination in selected cucurbitaceous crops

Crops such as, bitter gourd and oriental pickling melon were raised in 2.1 cents for each crop and the activity of pollinating bees were observed before and after the application of different plant protection measures at >50 per cent flowering of the crops. Observation on yield parameters were studied by selective exclusion of pollinating bees as control and compared with the yield of open pollinated plants. For these, two control treatments were maintained having three replications for each, where one control treatment was caged to observe the yield difference and the other was kept for open pollination by bees.

4.5.1 Number of bee visit to bitter gourd flowers by solitary pollen bees after spraying (post-monsoon-2018)

The results (Table 40) showed that there was a significant difference in bee visits to different treatments after a single day of spraying in the bitter gourd ecosystem in the post-monsoon-2018 season, in which plants treated with Azadirachtin 300 ppm (T_3) received the least number of bee visits (12.58) followed by Dimethoate 30 % EC (T_1) treated plants (13.91). The plants treated with Mancozeb 75 WP (T_5) and, Carbendazim 12 WP + Mancozeb 63 WP (T_6) were on par with each other. The highest number of bee visit was received in the untreated control (T_7) with a mean bee visit of 17.08. The third day after spraying was also found significantly different with respect to the bee visits in which, Azadirachtin 300 ppm (T_3) followed by Imidacloprid 200 SL (T_2) was on par with each other with a mean bee visit of 12.41 and 12.75 respectively. The highest mean number of bee visit on third day after spraying was observed in the untreated control (T_7) with an average of 18.00. The least mean number of bee visit on 5^{th}, 7^{th} and 9^{th} day after spraying was recorded in the Imidacloprid 200 SL (T_2) treated plants with an average of 12.00, 10.91 and, 12.16 respectively. The number of visit to Azadirachtin 300 ppm (T_3) treated plants found increased from the 7^{th} day after spraying. The highest number of bee visit was observed to be the highest in the untreated control up to the 15^{th} day after spraying.

Table 40. Average number of bee visit to bitter gourd flowers by solitary pollen bees after spraying in post-monsoon-2018

Treatment	1DBS	1DAS	3DAS	5DAS	7DAS	9DAS	11DAS	13DAS	15DAS
T_1	14.41^{b}	13.91^{cd}	13.33^{cd}	12.66^{bc}	13.83^{c}	13.50b^{c}	12.25^{b}	12.41^{b}	13.50^{bc}
T_2	17.25^{a}	14.58^{bc}	**12.75^{d}**	**12.00^{c}**	**10.91^{d}**	**12.16^{c}**	12.08^{b}	12.33^{b}	12.91^{c}
T_3	15.91^{ab}	**12.58^{d}**	**12.41^{d}**	13.00^{bc}	14.08^{bc}	13.75^{abc}	14.08^{ab}	15.16^{a}	14.83^{ab}
T_4	16.41^{a}	14.50^{bc}	15.66^{abc}	16.16^{a}	15.91^{ab}	15.33^{a}	15.83^{a}	15.50^{a}	16.25^{a}
T_5	17.16^{a}	16.16^{ab}	16.41^{ab}	16.16^{a}	15.66^{abc}	14.41^{ab}	15.41^{a}	15.08^{a}	15.50^{a}
T_6	16.58^{a}	15.75^{ab}	15.50^{bc}	14.66^{ab}	14.75^{abc}	14.41^{ab}	16.08^{a}	16.00^{a}	15.25^{ab}
T_7	17.08^{a}	**17.08^{a}**	**18.00^{a}**	**16.50^{a}**	**16.16^{a}**	**14.83^{ab}**	**15.41^{a}**	**14.91^{a}**	**15.58^{a}**
S.E.	0.56	0.56	0.75	0.70	0.60	0.58	0.70	0.65	0.57
C.D.	1.72	1.74	2.33	2.17	1.84	1.81	2.16	2.00	1.78

T_1: Dimethoate 30 % EC (@ 300 g ai ha^{-1}); **T_2:** Imidacloprid 200 SL (@ 30 g ai ha^{-1}); **T_3**: Azadirachtin 300 ppm (@ 0.03 %); **T_4**: *Beauveria bassiana* (@ 1 × 10^{8} spores ml^{-1}); **T_5**: Mancozeb 75 WP (@ 0.15%); **T_6**: Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2%); **T_7**: Untreated control

DBS: Day Before Spraying; **DAS**: Days After Spraying

4.5.2 Number of bee visit to bitter gourd flowers by solitary pollen bees after spraying (summer-2019)

The results (Table 41) showed that there was a significant difference in bee visit to different treatments after spraying different plant protection measures in bitter gourd ecosystem, in which Azadirachtin 300 ppm (T_3) treated plants received the least mean number of bee visit up to 15th day of spraying however, the number of bee visit got increased from the 9th day after spraying as compared to the 7th day after spraying. The highest mean number of bee visit was observed in the untreated control (T_7).

Table 41. Average number of bee visit to bitter gourd flowers by solitary pollen bees after spraying in summer-2019

Treatment	1DBS	1DAS	3DAS	5DAS	7DAS	9DAS	11DAS	13DAS	15DAS
T_1	25.16	23.00^{a}	22.50^{b}	25.33^{a}	23.33^{bc}	23.50^{cd}	24.33^{a}	23.25^{b}	23.16^{b}
T_2	24.16	20.41^{b}	21.16^{b}	22.58^{b}	22.91^{c}	23.16^{d}	24.00^{a}	23.91^{ab}	25.33^{a}
T_3	23.58	**15.00^{c}**	**15.16^{c}**	**17.50^{c}**	**17.91^{d}**	20.08^{e}	21.33^{b}	20.41^{c}	21.25^{c}
T_4	24.00	23.08^{a}	25.50^{a}	25.33^{a}	25.16^{abc}	25.33^{bc}	26.00^{a}	25.33^{ab}	25.41^{a}
T_5	24.66	23.25^{a}	26.08^{a}	24.83^{ab}	24.08^{abc}	25.41^{bc}	25.50^{a}	24.91^{ab}	25.00^{ab}
T_6	22.91	23.50^{a}	26.16^{a}	26.41^{a}	25.41^{ab}	26.41^{ab}	24.91^{a}	25.16^{ab}	25.75^{a}
T_7	22.75	**24.08^{a}**	**27.58^{a}**	**26.75^{a}**	**25.75^{a}**	**28.16^{a}**	**26.16^{a}**	**25.91^{a}**	**26.08^{a}**
S.E.	0.83	0.56	0.76	0.75	0.77	0.65	0.76	0.84	0.60
C.D.	NS	1.74	2.34	2.32	2.39	2.01	2.35	2.60	2.17

T_1: Dimethoate 30 % EC (@ 300 g ai ha^{-1}); **T_2:** Imidacloprid 200 SL (@ 30 g ai ha^{-1}); **T_3**: Azadirachtin 300 ppm (@ 0.03 %); **T_4**: *Beauveria bassiana* (@ 1 × 10^{8} spores ml^{-1}); **T_5**: Mancozeb 75 WP (@ 0.15%); **T_6**: Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2%); **T_7**: Untreated control

DBS: Day Before Spraying; **DAS**: Days After Spraying

4.5.3 Number of bee visit to bitter gourd flowers by solitary pollen bees after spraying (monsoon-2019)

The results (Table 42) showed that there was a significant difference in bee visit to different treatments after the first day, third day and fifth day after spraying in bitter gourd ecosystem, in which the least mean number of bee visit was observed in the Azadirachtin 300 ppm (T_3) treated plants in the 1st day and 3rd day after spraying with an average of 10.75 and 10.25 bees per plant respectively, whereas Dimethoate 30 % EC (T_1) received the least mean number of bee visit in the 5th day after spraying with an average of 10.66 bees per plant. The highest number of bee visit was observed in the untreated control on the 1st, 3rd and 5th day after spraying with an average of 13.50, 14.00 and 13.58 bees per plant. The average number of bee visit after spraying from the 7th day remained insignificant upto the 15th day of spraying.

Table 42. Average number of bee visit to bitter gourd flowers by solitary pollen bees after spraying in monsoon-2019

Treatment	1DBS	1DAS	3DAS	5DAS	7DAS	9DAS	11DAS	13DAS	15DAS
T_1	12.75	13.08^{a}	12.58^{b}	**10.66^{e}**	11.83	12.66	12.25	12.41	12.83
T_2	13.16	13.25^{a}	12.41^{b}	11.75^{cd}	11.58	12.16	12.58	13.08	12.50
T_3	12.58	**10.75^{c}**	**10.25^{c}**	11.66^{cd}	11.50	12.16	12.00	12.91	12.58
T_4	12.66	13.00^{a}	12.33^{b}	13.00^{ab}	12.08	13.00	12.50	12.58	12.41
T_5	12.41	12.66^{ab}	12.91^{ab}	12.50^{bc}	11.75	12.50	13.00	12.50	11.80
T_6	12.41	11.83^{b}	12.75^{b}	11.50d^{e}	12.75	12.41	12.83	12.91	12.75
T_7	13.00	**13.50^{a}**	**14.00^{a}**	**13.58^{a}**	13.83	13.66	13.41	13.16	13.25
S.E.	12.75	13.08^{a}	12.58^{b}	10.66^{e}	11.83	12.66	12.25	12.41	12.83
C.D.	NS	0.89	1.18	0.96	NS	NS	NS	NS	NS

T_1: Dimethoate 30 % EC (@ 300 g ai ha^{-1}); **T_2:** Imidacloprid 200 SL (@ 30 g ai ha^{-1}); **T_3**: Azadirachtin 300 ppm (@ 0.03 %); **T_4**: *Beauveria bassiana* (@ 1 × 10^{8} spores ml^{-1}); **T_5**: Mancozeb 75 WP (@ 0.15%); **T_6**: Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2%); **T_7**: Untreated control

DBS: Day Before Spraying; **DAS**: Days After Spraying

4.5.4 Number of bee visit to oriental pickling melon flowers by solitary pollen bees after spraying (Post-monsoon-2018)

The results (Table 43) showed that there was a significant difference in bee visit to different treatments after spraying in oriental pickling melon ecosystem in which, the least mean number of bee visit was observed in the Azadirachtin 300 ppm (T_3) treated plants with a mean bee visit of 24.00. The least mean number of bee visit on the 3rd day after spraying was observed in the Azadirachtin 300 ppm (T_3) plants with a mean number of 23.08 which was on par with the Imidacloprid 200 SL (T_2) treated plants which was recorded with an average bee visit of 24.50. On the 5th day after spraying, the least mean number of bee visit per plant was observed in the Azadirachtin 300 ppm (T_3) treated plants which were found on par with the Imidacloprid 200 SL (T_2) treated plants with an average bee visit of 24.25 and 24.50 respectively. The average number of bee visit was the least in the Azadirachtin 300

ppm (T_3) treated plants (26.25) on the 7th day after spraying followed by Dimethoate 30 % EC (T_1) treated plants (27.25) and Imidacloprid 200 SL (T_2) treated plants (27.66). Thus the treatments T_3, T_1 and T_2 were found to be on par with each other. The least average bee visit on the 9th and 11th day was recorded in the Azadirachtin 300 ppm (T_3) treated plants with a mean bee visit of 25.58 and 26.58 respectively. The average bee visit from the 11th day after spraying was not significantly different from each other.

Table 43. Average number of bee visit to oriental pickling melon flowers by solitary pollen bees after spraying in post-monsoon-2018

Treatment	1DBS	1DAS	3DAS	5DAS	7DAS	9DAS	11DAS	13DAS	15DAS
T_1	32.33	27.58^{b}	27.41^{bc}	27.67^{ab}	**27.25^{c}**	26.58^{bc}	28.75^{abc}	26.83	26.83
T_2	28.58	25.08^{cd}	**24.33^{d}**	**24.50^{c}**	**27.66^{c}**	26.16^{bc}	28.00^{bc}	25.16	26.91
T_3	31.58	**24.00^{d}**	**23.08^{d}**	**24.25^{c}**	**26.25^{c}**	**25.58^{c}**	**26.58^{c}**	26.50	26.25
T_4	31.41	29.33^{b}	28.25^{b}	28.25^{ab}	27.83^{bc}	27.08^{bc}	28.91^{abc}	27.25	28.33
T_5	31.00	27.25^{bc}	25.33^{cd}	26.16^{bc}	28.25^{bc}	26.83^{bc}	**26.91^{c}**	28.50	29.50
T_6	31.58	28.66^{b}	28.16^{b}	29.66^{a}	30.41^{ab}	28.50^{ab}	30.00^{ab}	29.08	29.25
T_7	31.41	**32.58^{a}**	**31.58^{a}**	**30.25^{a}**	**31.75^{a}**	**30.33^{a}**	**31.16^{a}**	29.75	29.17
S.E.	1.17	0.73	0.83	0.95	0.87	0.78	0.85	0.95	1.27
C.D.	NS	2.27	2.5	2.95	2.69	2.40	2.63	NS	NS

T_1: Dimethoate 30 % EC (@ 300 g ai ha^{-1}); **T_2:** Imidacloprid 200 SL (@ 30 g ai ha^{-1}); **T_3**: Azadirachtin 300 ppm (@ 0.03 %); **T_4**: *Beauveria bassiana* (@ 1×10^8 spores ml^{-1}); **T_5**: Mancozeb 75 WP (@ 0.15%); **T_6**: Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2%); **T_7**: Untreated control

DBS: Day Before Spraying; **DAS**: Days After Spraying

4.5.5 Number of bee visit to oriental pickling melon flowers by solitary pollen bees after spraying (summer-2019)

The results (Table 44) showed that there was a significant difference in bee visit to different treatments after spraying in oriental pickling melon ecosystem during summer (2019), where the least mean number of bee visit was observed in the

Imidacloprid 200 SL (T_2) with an average bee visit of 26.08 followed by Azadirachtin 300 ppm (T_3) treated plants with an average bee visit of 27.08 on the first day after spraying. Though the least average number of bee visit on the 3rd day after spraying was observed in the Azadirachtin 300 ppm (T_3) treated plants, it was found on par with the Imidacloprid 200 SL (T_2) treated plants with an average bee visits of 26.75 and 27.41 respectively. The average number of bee visit was lowest in the Imidacloprid 200 SL (T_2) treated plants on the 5th, 7th, 9th, 11th, 13th and, 15th day after spraying with an average bee visit of 26.91, 27.08, 25.66, 27.25, 28.00, 12.33 and, 26.25 respectively. The highest mean number of bee visit was observed in the untreated control.

Table 44. Average number of bee visit to oriental pickling melon flowers by solitary pollen bees after spraying in summer-2019

Treatment	1DBS	1DAS	3DAS	5DAS	7DAS	9DAS	11DAS	13DAS	15DAS
T_1	31.50^{abc}	28.66^{cd}	28.08^{c}	27.00^{d}	27.91^{c}	27.58^{bc}	29.08^{bc}	28.25^{b}	27.33^{cd}
T_2	33.58^{a}	**26.08^{e}**	**27.41^{c}**	**26.91^{d}**	**27.08^{c}**	**25.66^{c}**	**27.25^{c}**	**28.00^{b}**	**26.25^{d}**
T_3	30.33^{bc}	27.08^{de}	**26.75^{c}**	27.58^{cd}	27.75^{c}	29.83^{ab}	27.33^{c}	28.25^{b}	28.47^{bcd}
T_4	32.58^{ab}	30.16^{bc}	28.25^{bc}	29.83^{bc}	30.50^{b}	28.83^{ab}	27.33^{c}	30.75^{a}	29.66^{abc}
T_5	29.91^{c}	30.66^{bc}	29.00^{abc}	30.00^{b}	31.33^{ab}	28.83^{ab}	29.83^{ab}	32.25^{a}	30.41^{ab}
T_6	30.08^{c}	31.16^{ab}	31.00^{ab}	32.50^{a}	30.91^{b}	30.83^{a}	31.50^{a}	32.41^{a}	31.75^{a}
T_7	**33.25^{a}**	**33.25^{a}**	**31.66^{a}**	**34.33^{a}**	**32.75^{a}**	**31.08^{a}**	**31.83^{a}**	**32.58^{a}**	**32.00^{a}**
S.E.	0.74	0.69	0.92	0.76	0.56	0.58	0.70	0.65	0.57
C.D.	2.28	2.12	2.84	2.35	1.73	1.81	2.16	2.00	1.78

T_1: Dimethoate 30 % EC (@ 300 g ai ha^{-1}); **T_2:** Imidacloprid 200 SL (@ 30 g ai ha^{-1}); **T_3**: Azadirachtin 300 ppm (@ 0.03 %); **T_4**: *Beauveria bassiana* (@ 1 × 10^8 spores ml^{-1}); **T_5**: Mancozeb 75 WP (@ 0.15%); **T_6**: Carbendazim 12 WP + Mancozeb 63 WP (@ 0.2%); **T_7**: Untreated control

DBS: Day Before Spraying; **DAS**: Days After Spraying

4.5.6 Number of bee visit to oriental pickling melon flowers by solitary pollen bees after spraying (monsoon-2019)

The results (Table 45) showed that there was significant difference in bee visit to different treatments after spraying in oriental pickling melon ecosystem during monsoon-2019, where the least average number of bee visit was observed in Imidacloprid 200 SL (T_2) which was on par with Azadirachtin 300 ppm (T_3) with an average bee visit of 18.25 and 18.66 respectively. The 3rd and 5th days after spraying received the least average bee visit on the Azadirachtin 300 ppm (T_3) treated plants with an average of 17.25 for both days. The lowest average number of bee visit was recorded on the 7th and 9th day of the Azadirachtin 300 ppm (T_3) treated plants with an average bee visit of 19.50 and 20.08 respectively. However, this was found on par with Imidacloprid 200 SL (T_2) treated plants on both days. On the 11th day after spraying, the treatments T_1 (Dimethoate 30 % EC), T_2 (Imidacloprid 200 SL) and, T_3 (Azadirachtin 300 ppm) were observed to be on par with each other. On the 5th day after spraying, the plants treated with Dimethoate 30 % EC (T_1) and Azadirachtin 300 ppm (T_3) were found with the least average number of bee visit. The highest average number of bee visit was observed in the untreated control.

Table 45. Average number of bee visit to oriental pickling melon flowers by solitary pollen bees after spraying in monsoon-2019

Treatment	1DBS	1DAS	3DAS	5DAS	7DAS	9DAS	11DAS	13DAS	15DAS
T_1	23.16^{ab}	21.58^{ab}	21.91^{c}	22.08^{bc}	21.66^{b}	21.08^{de}	**22.08^{b}**	23.50^{bc}	**22.50^{c}**
T_2	21.83^{bc}	**18.25^{c}**	18.08^{d}	20.25^{d}	**19.58^{c}**	**19.58^{e}**	**21.00^{b}**	22.41^{cd}	24.58^{bc}
T_3	21.66^{c}	**18.66^{c}**	**17.25^{d}**	**17.25^{e}**	**19.50^{c}**	**20.08^{e}**	**20.83^{b}**	**21.41^{d}**	**22.50^{c}**
T_4	23.58^{a}	21.41^{b}	22.08^{bc}	20.41^{cd}	22.83^{ab}	22.16^{cd}	**22.08^{b}**	25.00^{ab}	25.16^{ab}
T_5	23.58^{a}	22.58^{ab}	23.00^{abc}	22.41^{b}	22.25^{b}	23.58^{bc}	**22.66^{b}**	24.41^{ab}	26.08^{ab}
T_6	22.41^{abc}	22.08^{ab}	23.33^{ab}	23.08^{ab}	23.00^{ab}	24.41^{ab}	25.16^{a}	25.25^{ab}	25.91^{ab}
T_7	23.66^{a}	**23.25^{a}**	**23.83^{a}**	**24.66^{a}**	**23.83^{a}**	**25.25^{a}**	**26.00^{a}**	**26.25^{a}**	**27.58^{a}**
S.E.	0.45	0.56	0.43	0.56	0.51	0.50	0.73	0.62	0.81
C.D.	1.39	1.74	1.33	1.74	1.57	1.54	2.25	1.92	2.49

4.5.7 Fruit set in bitter gourd in three different seasons

The fruit set in bitter gourd ecosystem was recorded for three different seasons *viz*., post-monsoon (2018), summer (2019) and, monsoon (2019) to assess the impact on fruit yield without pollination. In this experiment, treatments 1, 2, 3, 4, 5 and, 6 were compared to a caged control which was kept untreated with any plant protection measures. The highest yield from all three seasons was recorded in the Carbendazim 12 WP + Mancozeb 63 WP (T_6) treated plants *viz*., 2261.503 g, 2182.26 g and, 1761.95 g in post-monsoon (2018), summer (2019) and monsoon (2019) respectively. Whereas, the caged control was recorded with the lowest yield (Table 46) in all three seasons with an average fruit yield of 1107.91 g, 948.26 g and, 781.23 g in post-monsoon (2018), summer (2019) and monsoon (2019) respectively.

Table 46. Average fruit yield per plant of bitter gourd in three different seasons

Treatment	Yield per plant (g) post-monsoon (2018)	Yield per plant (g) summer (2019)	Yield per plant (g) monsoon (2019)
T_1	1693.18^{c}	1635.81^{b}	1672.10^{a}
T_2	1409.77^{e}	1360.98^{c}	1395.30^{c}
T_3	1416.91^{e}	1245.56^{c}	1198.73^{d}
T_4	1598.85^{d}	1313.83^{c}	1275.18^{d}
T_5	2106.52^{b}	1778.63^{b}	1517.19^{b}
T_6	2261.50^{a}	2182.26^{a}	1761.95^{a}
T_7	**1107.91^{f}**	**948.26^{d}**	**781.23^{e}**
S.E.	25.97	47.75	31.73
C.D.	80.92	148.77	98.87

4.5.8 Fruit set in oriental pickling melon in three different seasons

The fruit set in the oriental pickling melon ecosystem was recorded for three different seasons *viz*., post-monsoon (2018), summer (2019) and monsoon (2019) to assess the impact on fruit yield without pollination. In this experiment, treatments 1, 2, 3, 4, 5 and 6 were compared to a caged control which was kept untreated with any

plant protection measures. In post-monsoon (2018), the highest fruit yield recorded was in the Carbendazim 12 WP + Mancozeb 63 WP (T_6) treated plants with an average per plant fruit yield of 3920.58 g, which was followed by the Mancozeb 75 WP (T_5) with an average of 3822.11 g. In the summer (2019) season, the highest fruit yield per plant was obtained in the 5th treatment which was treated with the Mancozeb 75 WP with an average per plant yield of 3441.95 g, followed by the Carbendazim 12 WP + Mancozeb 63 WP and the *Beauveria bassiana* treated plants with an average per plant yield of 3323.08 g and 3320.21 g respectively. In monsoon (2019), the highest per plant fruit yield was recorded in the Mancozeb 75 WP (T_5) treated plants with an average of 3700.26 g, which was followed by *Beauveria bassiana* (T_4) with an average per plant fruit yield of 3666.40 g. The least average fruit yield per plant was recorded in the caged plants in all three seasons, with an average of 1023.37 g, 1031.73 g and 1011.73 g in post-monsoon (2018), summer (2019) and monsoon (2019) respectively (Table 47).

Table 47. Average fruit yield per plant of oriental pickling melon in three different seasons

Treatment	Yield per plant (g) post- monsoon (2018)	Yield per plant (g) summer (2019)	Yield per plant (g) monsoon (2019)
T_1	3536.17^{b}	2960.53^{b}	3110.30^{b}
T_2	2637.00^{c}	3177.85^{ab}	2983.92^{b}
T_3	2410.24^{c}	3112.28^{ab}	2942.00^{b}
T_4	3532.13^{b}	3320.21^{ab}	3666.40^{a}
T_5	3822.11^{a}	3441.95^{a}	3700.26^{a}
T_6	3920.58^{a}	3323.08^{ab}	3528.50^{a}
T_7	**1023.37^{d}**	**1031.73^{c}**	**1011.73^{c}**
S.E.	140.71	92.71	79.83
C.D.	438.37	288.84	248.71

Discussion

5. DISCUSSION

The discussion on results obtained from the study on "Pollination ecology of solitary pollen bees" conducted at the Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellanikkara are summarized in this chapter to elucidate the various observations and findings.

5.1 Documentation of pollinator diversity and relative abundance of all pollinators in selected cucurbitaceous crops

In the present study, a total of 45 species of flower visitors were recorded in bitter gourd and oriental pickling melon fields belonging to 11 families of 3 orders *viz.*, Hymenoptera, Diptera and Lepidoptera from the roving survey conducted from March 2018 to December 2019.

Among the flower visitors, 91.11 per cent of species belonged to the order Hymenoptera, followed by 4.44 per cent of species of Diptera and 4.44 per cent of species of Lepidoptera. Mevetty *et al.* (1989) pointed out that the density of insects on flowers depended upon several factors such as flower shape, size, colour, availability of floral rewards and weather conditions, which supported the variation in the relative abundance of flower visitors during the period of the survey. In the present survey conducted from March 2018 to December 2019, the relative abundance of pollinators varied from 0.12 per cent to 18.05 per cent, which could be due to the weather conditions, floral resources, differences in the environmental conditions and uniqueness of areas surveyed (Figure1). Singh *et al*. (2004) opined that the maximum number of floral visitors was observed during the mid-morning hours to the afternoon which was in concordance with the present study.

Our present study revealed that the major chunk of pollinators of cucurbits belonged to the order Hymenoptera, which included *Braunsapis picitarsis* (Cameron), *Braunsapis mixta* (Smith), *Ceratina smaragdula* (F.), *Ceratina binghami* Cockerell, *Ceratina hieroglyphica* Smith, *Ceratina* sp., *Xylocopa ruficornis* Fab., *Xylocopa fenestrata* Fab., *Amegilla zonata* (L.), *Thyreus* sp. 1, *Thyreus* sp. 2, *Apis cerana* Fab., *Apis florea* Fab., *Apis dorsata* Fab., *Tetragonula iridipennis* Smith., *Halictus* sp., *Lasioglossum serenum* (Cameron), *Nomia curvipes* (Fab.), *Hoplonomia elliotti* (Smith), *Gnathonomia thoracica* Smith., *Leuconomia interstitialis* Cameron, *Nomiapis* sp., *Lipotriches* sp., *Coelioxys* sp., *Megachile disjuncta* (Fab.), *Megachile*

sp. 1, *Megachile* sp. 2, *Antodynerus punctatipennis* (de Saussure), *Delta pyriforme* (Fabricius), *Eumenes macrops* de Saussure, *Phimenes flavopictus* (Blanchard), *Rhynchium brunneum* (Fabricius), *Ropalidia brevita* Das & Gupta, *Phalerimeris phalerata turneri* (Betrem), *Scolia affinis* (Guerin), *Scolia cyanipennis* Fabricius, *Chalybion bengalense* (Dahlbom), *Sphex argentatus* Fabricius*, Sphex sericeus* (Fabricius), *Chrysis* sp. and *Tachytes* sp. The remaining species (*Chrysomya* sp., *Syritta* sp. *Eurema* sp*., and Acytolepis* sp.) were rarely caught in sweep net collections during the survey period. Similar observations were made by Deyto and Cervanica (2009), where they found that insects visiting the bitter gourd flowers belonged to four insect orders *viz*., Hymenoptera (*Apis cerana* Fabricius, *Apis mellifera* Linnaeus, *Xylocopa* spp., *Trigona* spp. and *Halictus* spp.), Diptera (*Calliphora* sp., Sarcophagidae and Syrphidae), Coleoptera (Chrysomelidae) and Lepidoptera (butterflies). Subhakar *et al*. (2011) reported a total of 17 pollinator species belonging to four orders *viz.*, Hymenoptera, Diptera, Coleoptera, and Lepidoptera, where *Tetragonula iridipennis* (Smith), *Halictus gutturosus* Vachal and *Apis florea* Fabricius were the most abundant pollinators, which was in concordance with our study, in which *T. iridipennis* (18.05 %) was reported as the predominant cucurbit pollinator based on the relative abundance from the sweep net collection followed by *A. cerana* (17.07 %) and *A*. *florea* (15.11 %), whereas the relative abundance of *Halictus* sp. among all the pollinators was very less in cucurbit ecosystems of Central Kerala. Though the relative abundance of *Lasioglossum* sp. and *Xylocopa* spp. were low in the sweep net collection from cucurbitaceous ecosystems of central Kerala, Oronje, *et al*. (2012) observed that *A*. *mellifera, Lasioglossum* sp. and *Xylocopa* spp. were efficient pollinators of bitter gourd. Tharini (2016) had similar observations on bitter gourd pollinators which supported the current study, where 27 species of flower visitors belonging to seven families and three orders (Hymenoptera, Diptera, and Lepidoptera) were recorded.

In the present study, it was found that non-*Apis* bees (65.10 %) were the most abundant flower visitors of selected cucurbit crops as compared to *Apis* bees (35.25 %) in the sweep net collection during the roving surveys. But according to Tharini (2016), *Apis* bees were more abundant flower visitors in bitter gourd when compared to the other non-*Apis* bees in the field study.

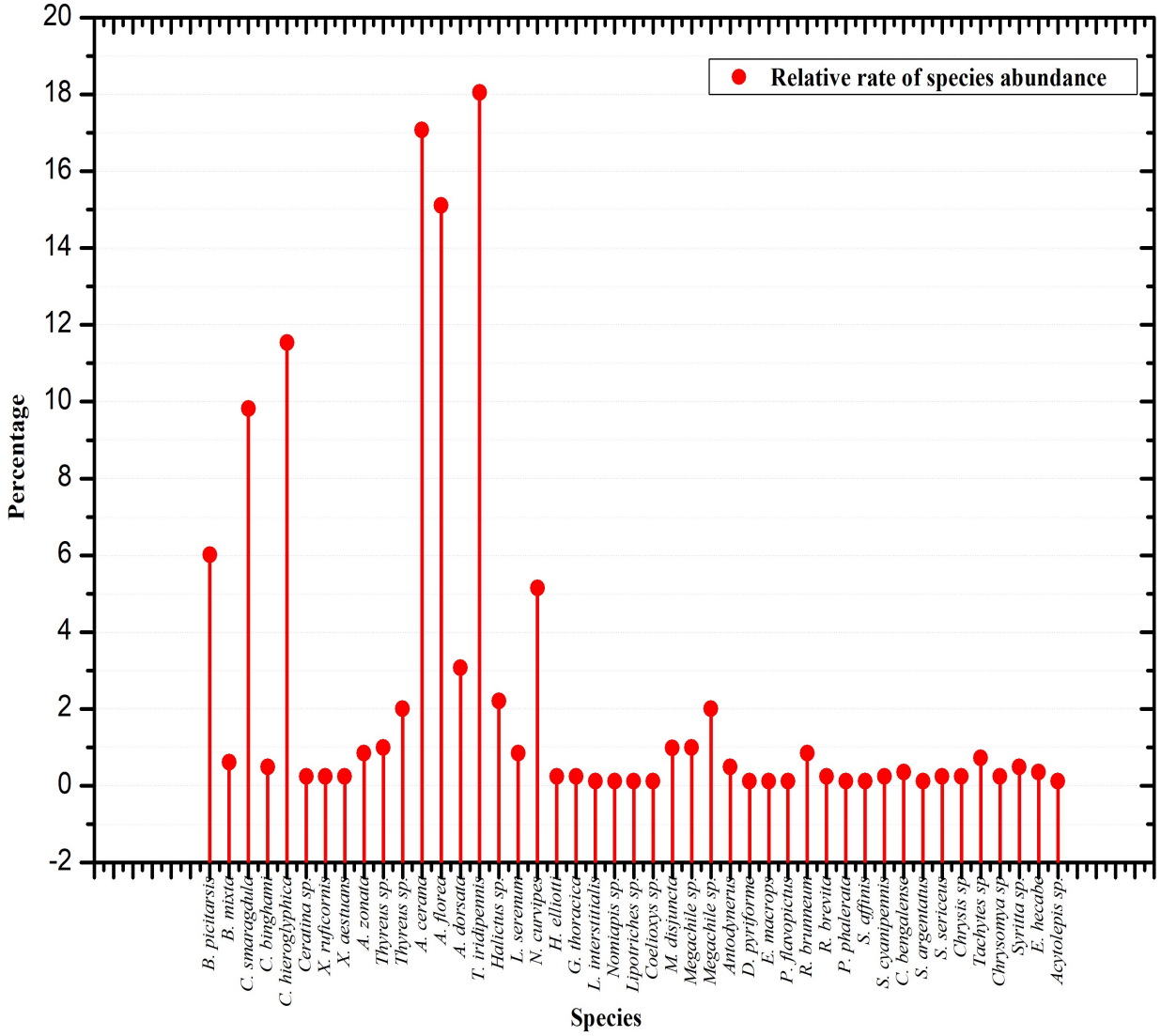


Figure 1. Relative rate of species abundance recorded in the roving survey conducted from March 2018 to December 2019

Bitter gourd and oriental pickling melon were found to be highly cross-pollinated crops and thus required small bees for the effective transfer of pollen from male to female flowers (Sands, 1928). In the present study, among the hymenopteran pollinators, 95.64 per cent were bees, whereas 4.35 per cent were wasps. This confirmed the fact that bees were the most efficient pollinators of cucurbit ecosystems as compared to the other pollinator fauna. Alex (1957) also reported that honey bees were the most abundant group of pollinators in cucurbits and also pointed out that ants, thrips, beetles, and solitary bees could also be possible pollinators of cucurbits. The present study revealed that *T. iridipennis* was the predominant pollinator of bitter gourd and oriental pickling melon flowers followed by *A. cerana*, *A. florea*, *C. hieroglyphica, C. smaragdula,* and *B. picitarsis.* Saeed *et al.* (2012) also reported *A. cerana*, *A. florea*, *C. sexmaculata,* and *Lasioglossum* sp. as the major pollinators of bitter gourd flowers. Balina *et al.* (2012) observed that *Halictus* sp., *Megachile* sp. and, *A. dorsata* were the major floral visitors of bitter gourd in Haryana. Yogapriya *et al.* (2019) had similar observations to that of our present study, in which they recorded *T. iridipennis* as the major pollinator of bitter gourd followed by *A. florea*, *Halictus* sp. and, *A. cerana.* Similarly, Bisui *et al.* (2020) reported *Halictus* sp. as the major pollinator of bitter gourd flowers followed by *A. dorsata*, *Lasioglossum* sp., *A. cerana* and, *T. iridipennis*.

5.1.1. Diversity indices of all pollinators in the selected cucurbitaceous ecosystems of Central Kerala

In the present study, diversity indices *viz*., Simpson's diversity index (1-D), Shannon-Weiner index (H'), Brillouin index (H_B), Berger-Parker index, Menhinick's index, Margalef's index, and Pielou's evenness (J) index were measured to study the diversity, richness, and evenness of the pollinators collected from three districts of Central Kerala. Simpson's diversity index (1-D), Shannon's diversity index (H'), and Brillouin's index were high in the Thrissur district compared to the Palakkad and Ernakulam districts which showed that the species diversity was high in the Thrissur district.

Widhiono *et al*. (2017) studied the insect visitation to agricultural ecosystems of central Java and observed that species diversity increased with increasing elevation. In the present study, three selected districts in Kerala were a

combination of lowland, midland, and, highland, wherein species recorded did not show a wide range in distribution except the fact that Palakkad district was rich in several singleton and doubleton species which were specific to the district as those were not caught in sweep net collections from the other two districts. Most of the solitary bee species were collected from the cucurbit fields of Palakkad, where the farmers followed more eco-friendly plant protection measures such as less usage of chemical pesticides, maintaining field flora and organic control of insect pests. This might have contributed to more species diversity of non-*Apis* bees in the Palakkad district.

Baboo (2020) conducted a study to record the diversity of native insect pollinators in three districts *viz*., Kozhikode, Malappuram, and Wayanad, and found that the frequency of insect visitors was the highest during post-monsoon in lowlands and highlands while it was higher in monsoon in midlands based on the Shannon diversity index and evenness index. In the present study, the diversity indices (*H', H_B* and 1-D) were high in the Thrissur district followed by the Palakkad and Ernakulam districts. Species diversity was comparatively low in the Ernakulam district in the sweep net collection but the Berger-Parker index was high in the Ernakulam district. The Berger-Parker index showed that among the insect pollinator species collected from the Ernakulam district, one species was the most dominant which contributed to a higher index, whereas the insect pollinators collected from the other two districts were uniformly distributed.

5.1.2. Molecular characterization of solitary pollen bees

Molecular characterization of different species of solitary pollen bees coming under the same genera was done to confirm their identity. A total of 23 solitary pollen bees were recorded during the roving survey conducted in the selected cucurbitaceous ecosystem, out of which DNA barcoding of 15 species was done to confirm their identity.

The adults of allodapine bee, *B. picitarsis* are highly cryptic and closely resemble *B. mixta* and hence the specimens collected were subjected to DNA barcoding to confirm the species identity. Kaliaperumal *et al.* (2022) conducted a similar study in which they identified their specimen as *B. mixta* as they showed 100 per cent similarity in identity to the corresponding species at BLASTn search tool of

NCBI. The specimen was then submitted to NCBI and accession numbers for the specimen were generated (MW135190, MW619047, MZ619047, MZ619048 and, MZ619049). Similarly, *B. picitarsis* and *B. mixta* specimens collected during the present study were submitted to NCBI GenBank database, and accession numbers, MW856777 and MW856776 were generated respectively. The specimens were also submitted to the BOLD systems to generate barcodes and BIN numbers (*B. picitarsis*; BOLD: AET3422 and, *B. mixta*; BOLD: AEU4376) were generated.

Bhat *et al.* (2022), conducted molecular characterization of three small carpenter bees in the north-western Indian Himalayas and confirmed their identity as, *Ceratina smaragdula*, *C. sutepensis* and *C. similima.* In the present study, the molecular characterization of three *Ceratina* bees was done to confirm their species identity. Three specimens were identified as *C. smaragdula*, *C. hieroglyphica*, and *C. binghami* which were submitted to NCBI GenBank and also to BOLD systems V3 to generate respective accession numbers and BIN numbers.

5.2 Determination of peak foraging time of solitary pollen bees

The data on the mean number of solitary pollen bees per square meter area at hourly intervals were recorded from 6.00 AM to 12.00 PM starting from the 50 per cent flowering period of bitter gourd and oriental pickling melon crops.

In bitter gourd, the mean number of solitary pollen bees per square meter area (N=100 days) was the highest during the hour, 9.00 AM to 10.00 AM during post-monsoon 2018 (446±40.9), summer 2019 (467.2±33.5) and monsoon 2019 (353±22.6) seasons. Whereas, the least mean number of solitary pollen bees were recorded during the hour 6.00 AM to 7.00 AM in all three seasons *viz*., post-monsoon 2018 (206.4±29.7), summer 2019 (205.8±27.5) and, monsoon 2019 (124.2±12.9) (Figure 2). A study conducted by Subhakar and Sreedevi (2015) on pollinators of bitter gourd found that the sweat bee, *Halictus gutturosus* Vachal started their foraging activity by 07.00 AM and their foraging activity increased upto 10.00 AM with a maximum bee activity at 09.00 AM. While they also reported that the activity of *H. gutturosus* declined from 10.00 AM onwards with a minimum activity at 1.30 PM. These findings were in concordance with the current study where all the solitary bee species appeared to be at their highest activity from 9.00 AM to 10.00 AM and afterward, the activity got reduced.

In oriental pickling melon, the mean number of solitary pollen bees per square meter area (N=100 days) was the highest during the hour, 9.00 AM to 10.00 AM during post-monsoon 2018 (369.4±16.4) and monsoon 2019 (343.8±27.6) seasons. However, the highest mean number of solitary pollen bees per square meter area in summer-2019 was during the hours 10.00 AM to 11.00 AM (386.6±20.2). The least mean number of solitary pollen bees were recorded during the hour 11.00 AM to 12.00 PM in all three seasons *viz*., post-monsoon 2018 (177.6±14.1), summer 2019 (168.4±14.3) and, monsoon 2019 (186.8±13.6) (Figure 3). In the oriental pickling melon ecosystem, except for the summer-2019, peak activity of solitary pollen bees were noticed at 9.00 AM to 10.00 AM and later their activity got reduced. Vijayakumar *et al.* (2022) reported the polylectic solitary pollen bee, *Hoplonomia westwoodi* from crops such as cucumber, tomato, brinjal, chilli, okra, *etc* where the bee started their foraging activity by 6.00 to 6.30 AM and reached the peak of their activity by 10.00 AM and their foraging activity declined after 1.00 PM. These findings were under the current study in which solitary bees in oriental pickling melon reached their peak activity at 9.00 AM to 10.00 AM and got gradually decreased after 11.00 AM.

In the present study conducted for determining the peak foraging activity of solitary bees in bitter gourd and oriental pickling melon ecosystems, the higher activity of solitary bees was observed upto 11.00 AM. This might be attributed to the anthesis, anther dehiscence, nectar flow and availability of pollen in the gourd flowers. The foraging activity got reduced after 1.00 PM which might be due to the closure of gourd flowers.

5.2.1 Abundance of flower visitors at 100 per cent flowering in bitter gourd ecosystem during three different seasons (post-monsoon-2018, summer-2019 and monsoon-2019)

The visual counts on all flower visitors to their abundance at 100 per cent flowering stage in bitter gourd ecosystem showed that, *T. iridipennis* was the most abundant pollinator followed by *A. cerana*, *B. picitarsis* and, *C. hieroglyphica* in all three seasons (Figure 4). *B. picitarsis* was the most abundant pollinator among the solitary pollen bees observed under the bitter gourd ecosystem. Balachandran *et al.* (2017) reported stingless bee, *Trigona* sp. as the major pollinator of bitter gourd

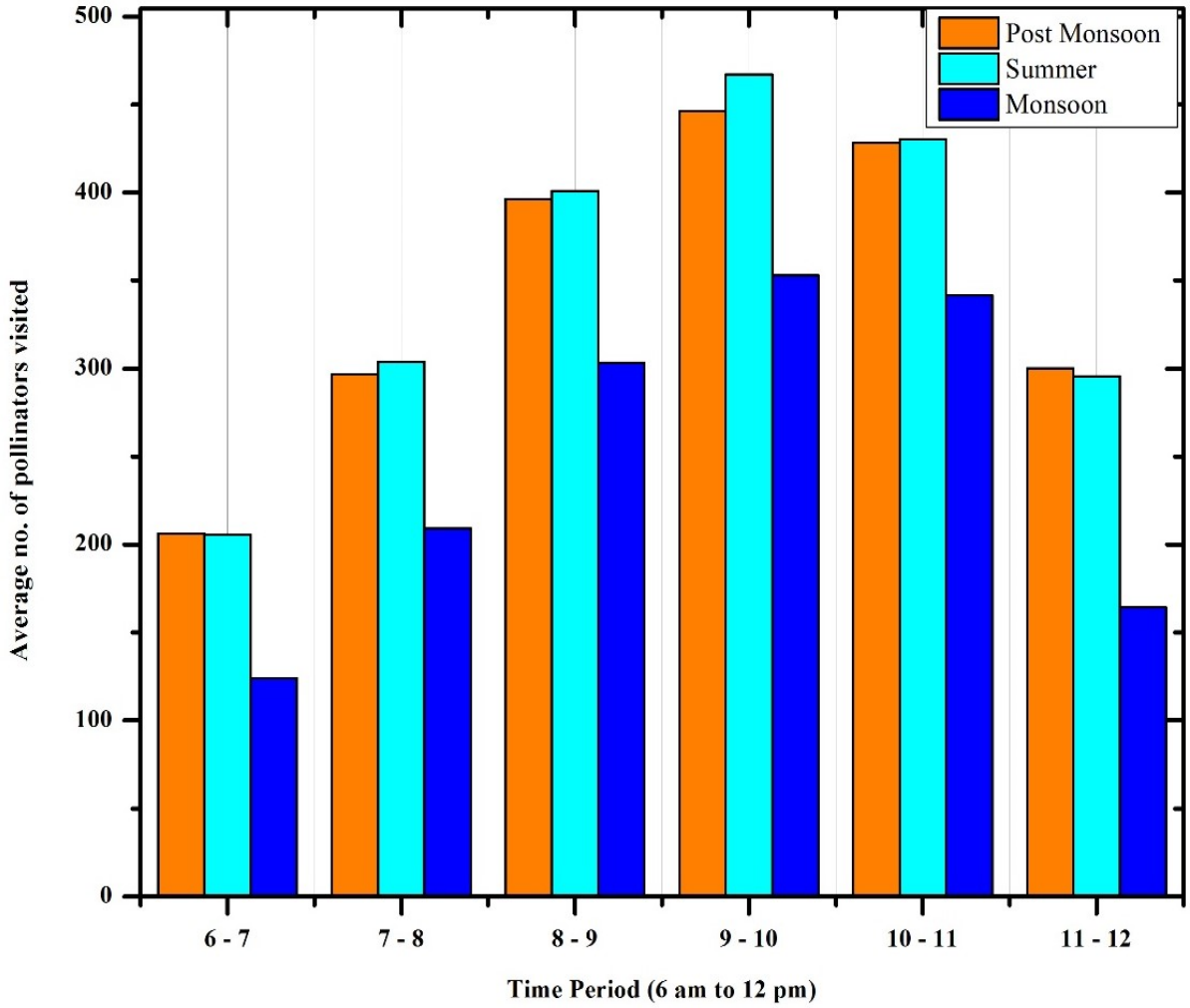


Figure 2. Mean number of solitary pollen bees per square meter area in three different seasons in bitter gourd ecosystem at six different time intervals

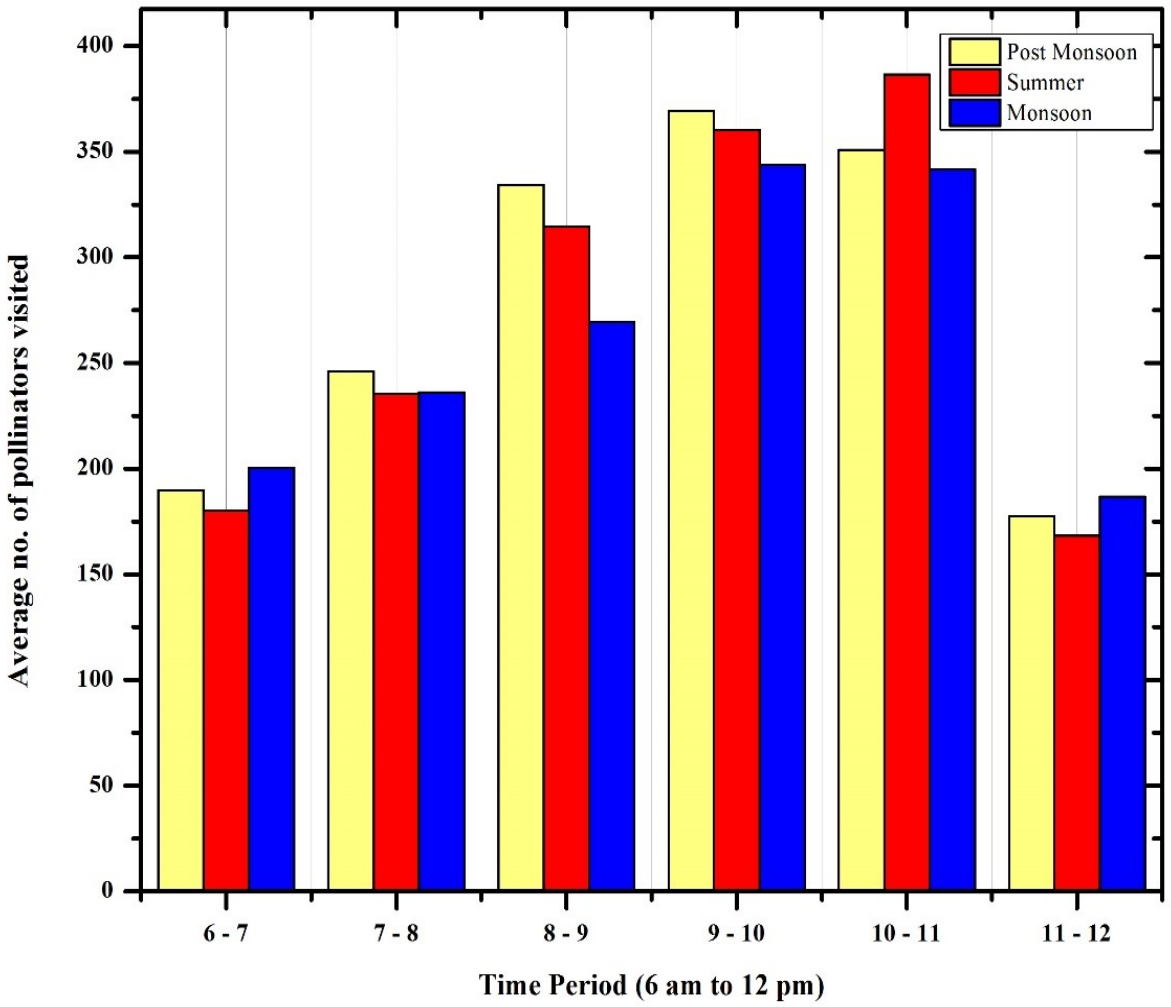


Figure 3. Mean number of solitary pollen bees per square meter area in three different seasons in oriental pickling melon ecosystem at six different time intervals

which had their peak visitations at 9.30 AM and their least number of visitations at 7.30 AM.

5.2.2 Abundance of flower visitors at 100 per cent flowering in the oriental pickling melon ecosystem during three different seasons (post-monsoon 2018, summer 2019 and monsoon 2019)

The visual counts on all flower visitors to their abundance at 100 per cent flowering stage in the oriental pickling melon ecosystem showed that, *T. iridipennis* was the most abundant pollinator followed by *A. cerana* in all three seasons. (Figure 5). *B. picitarsis* (14.79 %) was the most abundant pollinator among the solitary bees during post-monsoon-2018, followed by *C. hieroglyphica* (14.20 %) and, *C. smaragdula* (5.19 %). Whereas, summer 2019 season indicated *C. hieroglyphica* as the most abundant pollinator (13.52), followed by *B. picitarsis* (11.80 %) and *C. smaragdula* (11.61 %). *C. hieroglyphica* was the most abundant with a per cent abundance of 17.53 in monsoon-2019, followed by *C. smaragdula* (14.38 %) and *B. picitarsis* (9.86 %). The per cent abundance of the small carpenter bee, *C. smaragdula* was found the highest in the monsoon season, whereas the lowest was in post-monsoon season, and this might be due to their overwintering behavior during post-monsoon season. In the present study, *C. smaragdula* was observed to be a major pollinator under solitary pollen bee species of the oriental pickling melon ecosystem.

5.2.3. Number of visit by pollinators in bitter gourd ecosystem at different per cent flowering in three different seasons

The average number of visit by pollinators in the bitter gourd ecosystem at 25, 50, 75 and >90 per cent flowering was observed (N=5 days) to record the pollinator species with the maximum number of visits to gourd flowers. *T. iridipennis* was found as the pollinator species to give a maximum number of visits to bitter gourd flowers in three seasons at four different flowering percentages. *B. picitarsis* was the solitary bee species to give maximum flower visit to bitter gourd flowers in three different seasons at four different flowering percentages (Figure 6 - post-monsoon - 2018; Figure 7- summer-2019; Figure 8 - monsoon-2019). The number of visit by all pollinators increased with an increase in per cent flowering in all three seasons. This might be attributed to the factors like increased pollen and nectar resources with an increased flowering percentage.

5.2.4. Number of visit by pollinators in oriental pickling melon ecosystem at different per cent flowering in three different seasons

The average number of visit by pollinators in the oriental pickling melon ecosystem at 25, 50, 75 and >90 per cent flowering was observed (N=5 days) to record the pollinator species with a maximum number of visit to gourd flowers. *T. iridipennis* was found as the pollinator species to give the maximum number of visit to bitter gourd flowers in three seasons at four different flowering percentages. *C. hieroglyphica*, *B. picitarsis* and *C. smaragdula* were the solitary bee species to give maximum flower visit to oriental pickling melon flowers in three different seasons at four different flowering percentages (Figure 9 - post -monsoon - 2018; Figure 10 - summer - 2019; Figure 11 monsoon - 2019). The number of visit by all pollinators increased with an increase in per cent flowering in all three seasons. This might be due to the increase in pollen availability and nectar resources along with an increase in flowering percentage.

5.3 Studying the nesting preferences of solitary pollen bees

The recent declines in bee populations along with the increase in demand for pollination services have led to the development of strategies to increase and attract native bee pollinators (Olsson *et al*., 2015). As bees need two basic resources *i.e*., food and nesting habitat, the proximity of nesting habitat and floral resources could increase the diversity of native bee pollinators (Holzschuh *et al*., 2012). Rahimi *et al*. (2021) suggested that determination of the nesting habitat of bees could be difficult as different species of bees required different nest habitats. Identification of the nest type of bees was critical for attracting these species to the concerned ecosystems (Bennett and Lovell, 2019). One of the best strategies to attract pollinators to the ecosystems was to provide artificial nests to study, monitor and increase the bee populations (Leonard and Harmon-Threatt, 2019). In the present study, artificial nesting sites were established with 20 specific nest hole sizes and two nesting substrates and were placed at three different distances away from the field to study the nesting preferences of major solitary bee pollinators of selected cucurbit crops *i.e.,* bitter gourd and oriental pickling melon. The per cent nest occupancy of solitary bee nesters as well as other arthropod nesters were recorded monthly for three consecutive years *i.e.,* 2019, 2020 and 2021. A three-factor factorial analysis was made to assess the effect of different

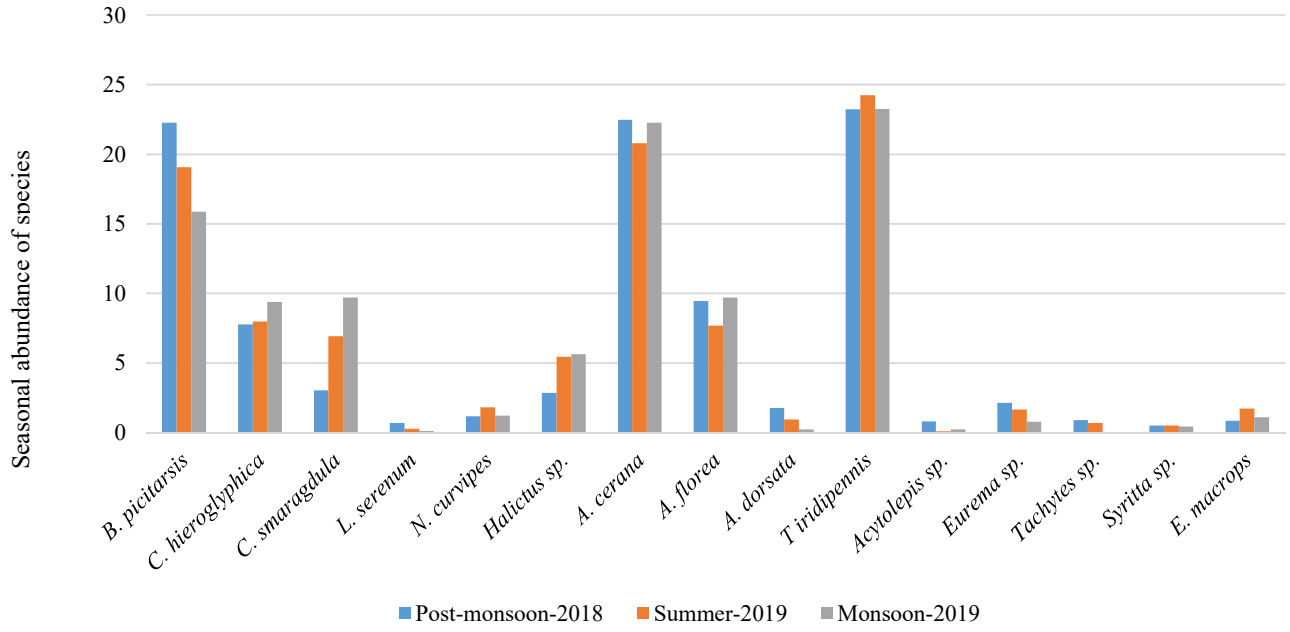


Figure 4. Abundance of flower visitors at 100 per cent flowering in the bitter gourd ecosystem during three different seasons (post-monsoon 2018, summer 2019 and monsoon 2019)

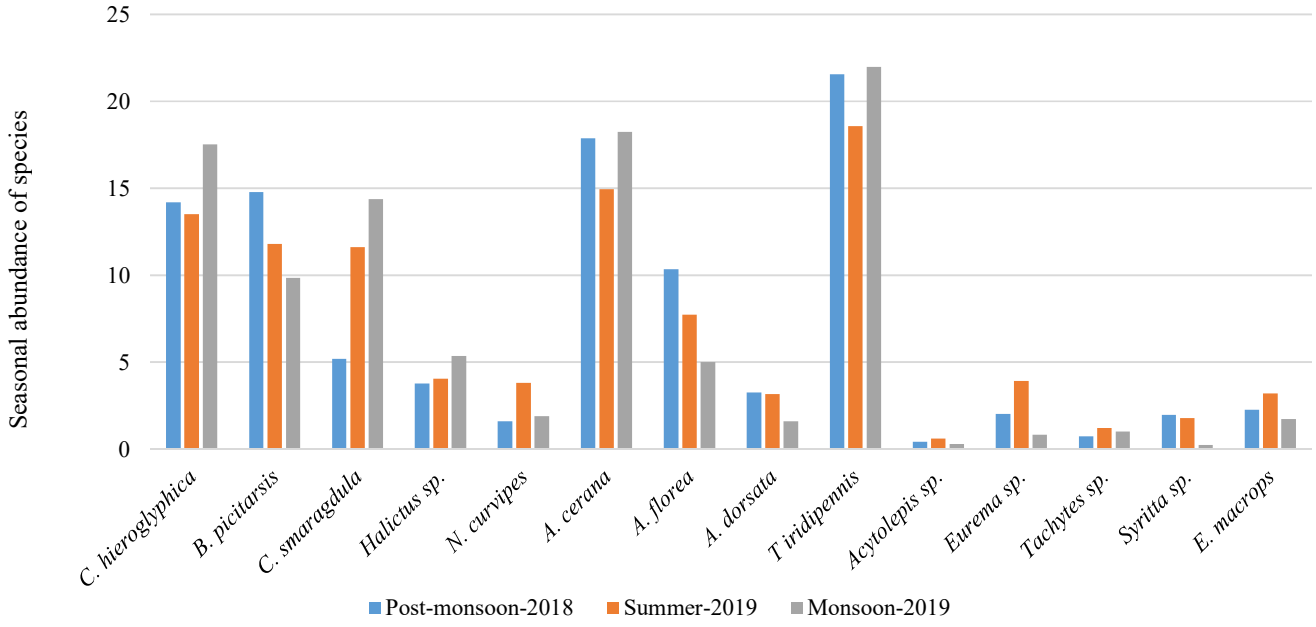


Figure 5. Abundance of flower visitors at 100 per cent flowering in oriental pickling melon ecosystem during three different seasons (post-monsoon-2018, summer-2019 and monsoon-2019)

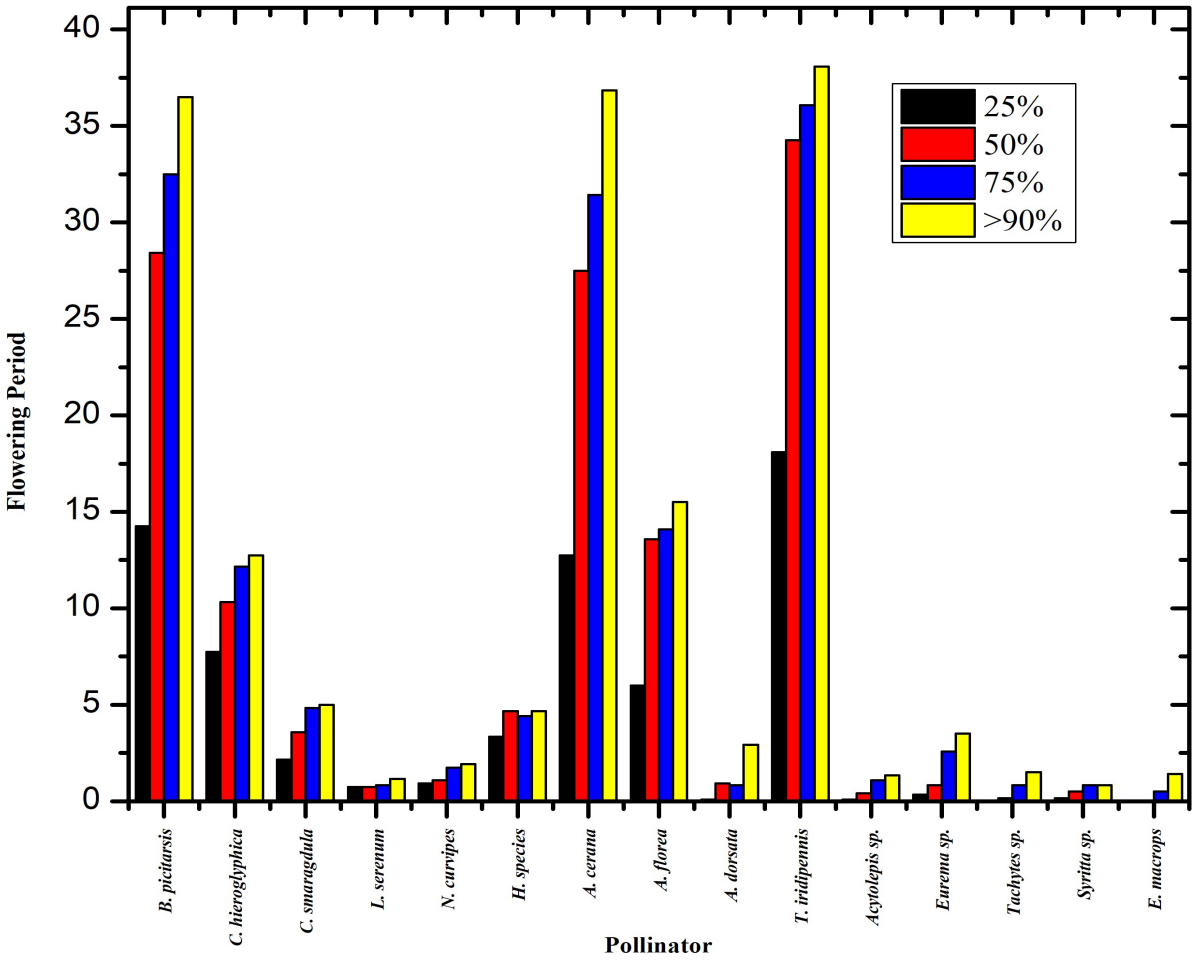


Figure 6. Average number of visit by pollinators in bitter gourd ecosystem at different per cent flowering during post-monsoon-2018

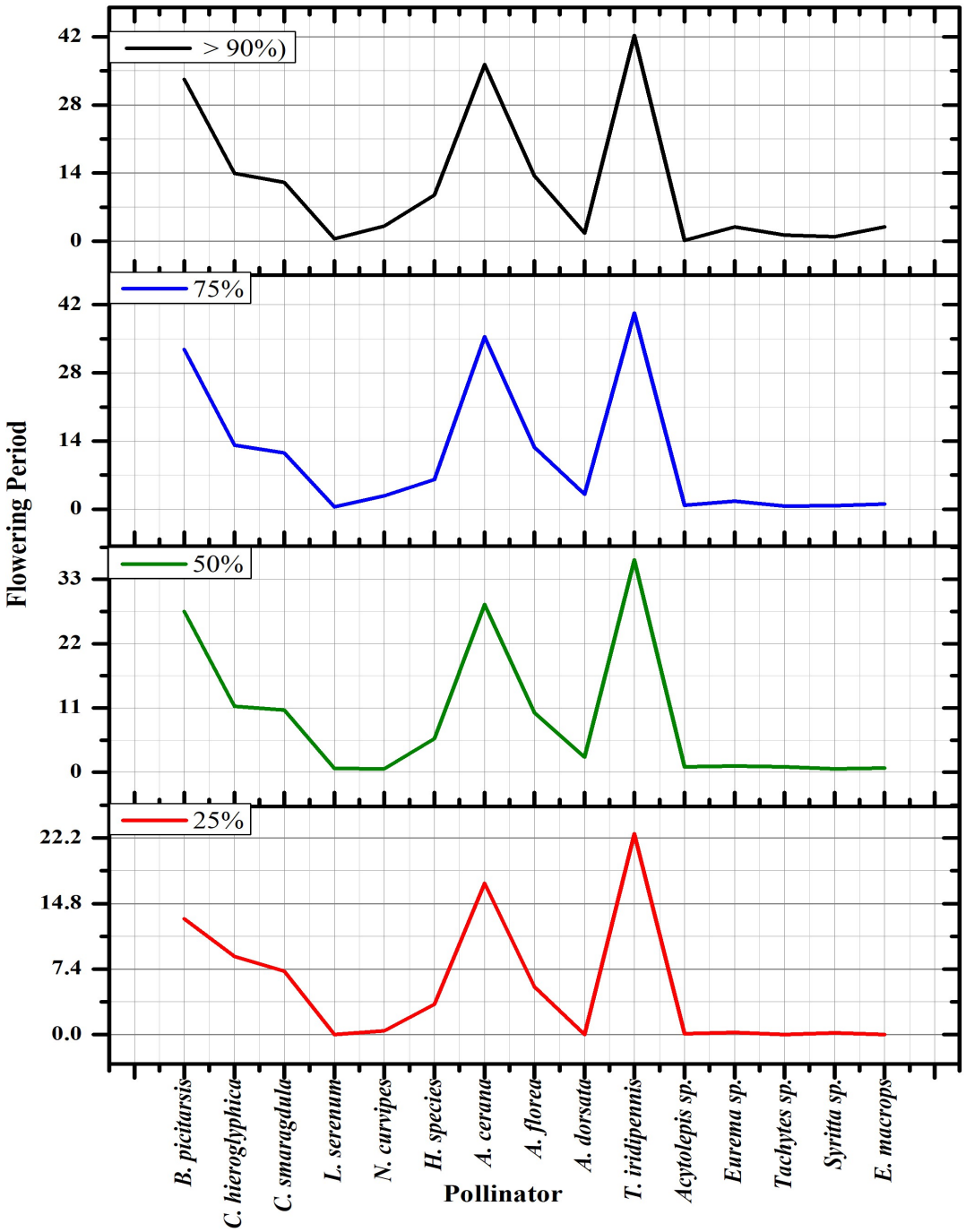


Figure 7. Average number of visit by pollinators in bitter gourd ecosystem at different flowering per cent during summer-2019

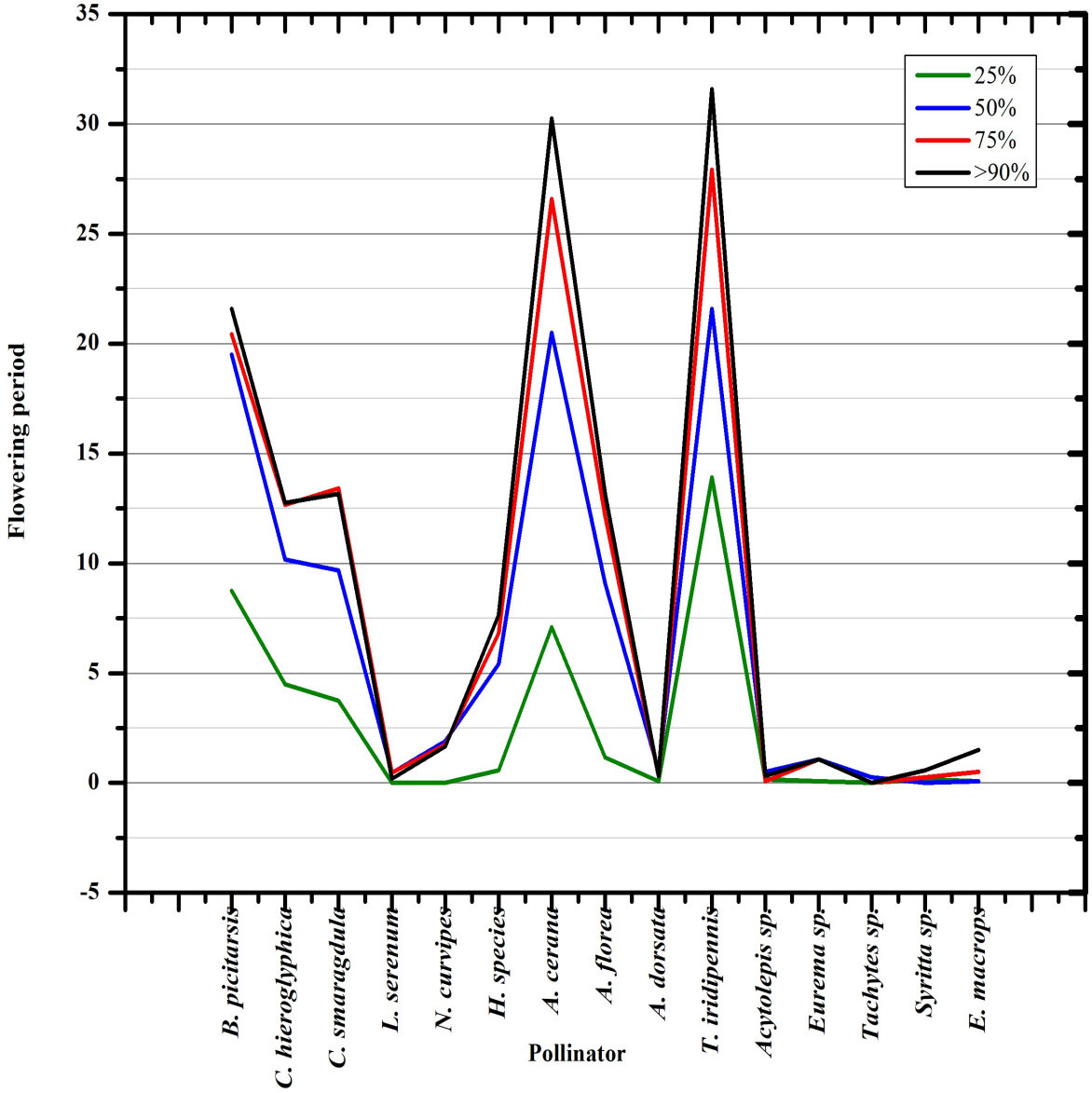


Figure 8. The average number of visit by pollinators in the bitter gourd ecosystem at different flowering per cent during monsoon-2019

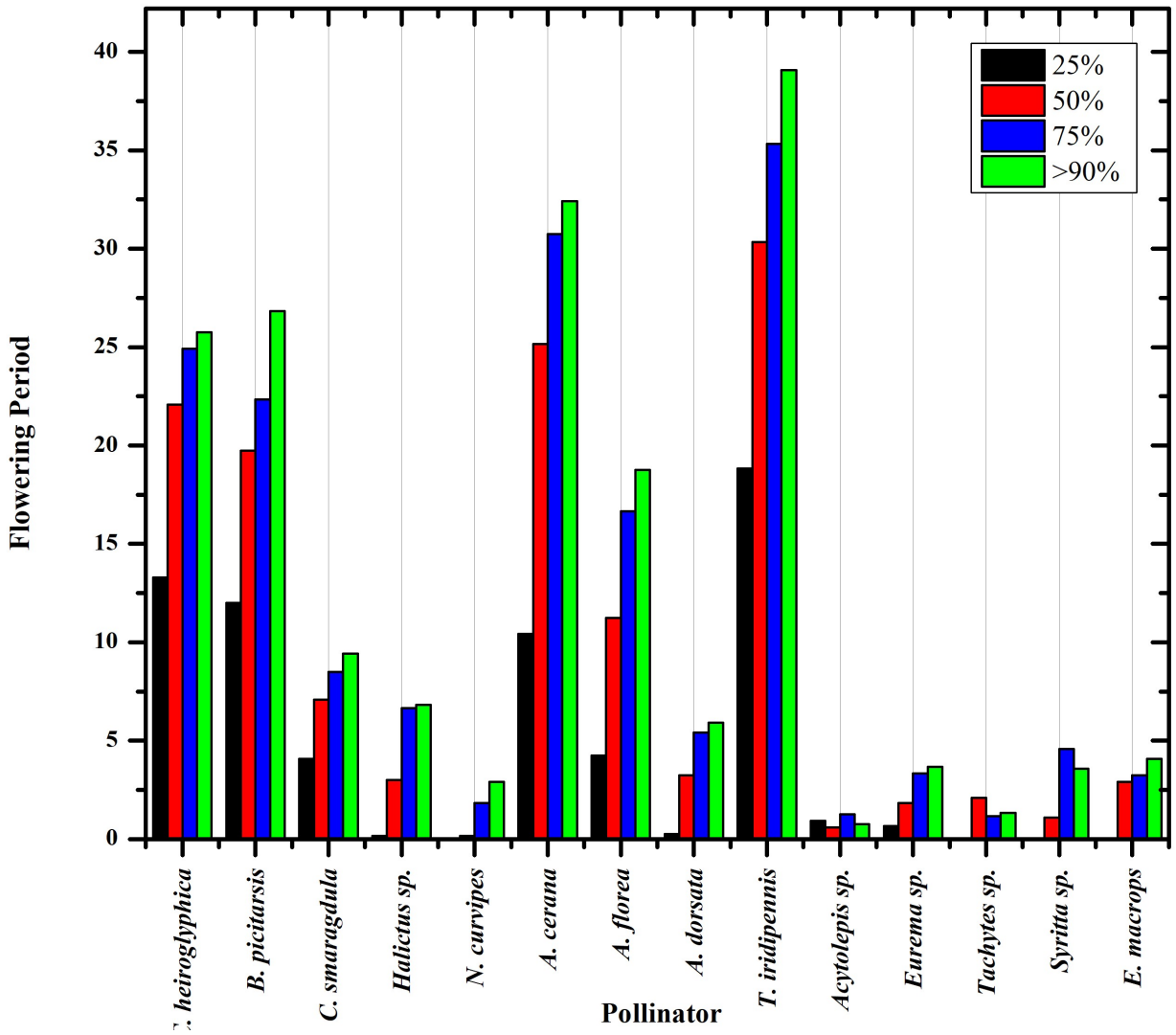


Figure 9. Average number of visit by pollinators in oriental pickling melon ecosystem at different per cent flowering during post-monsoon-2018

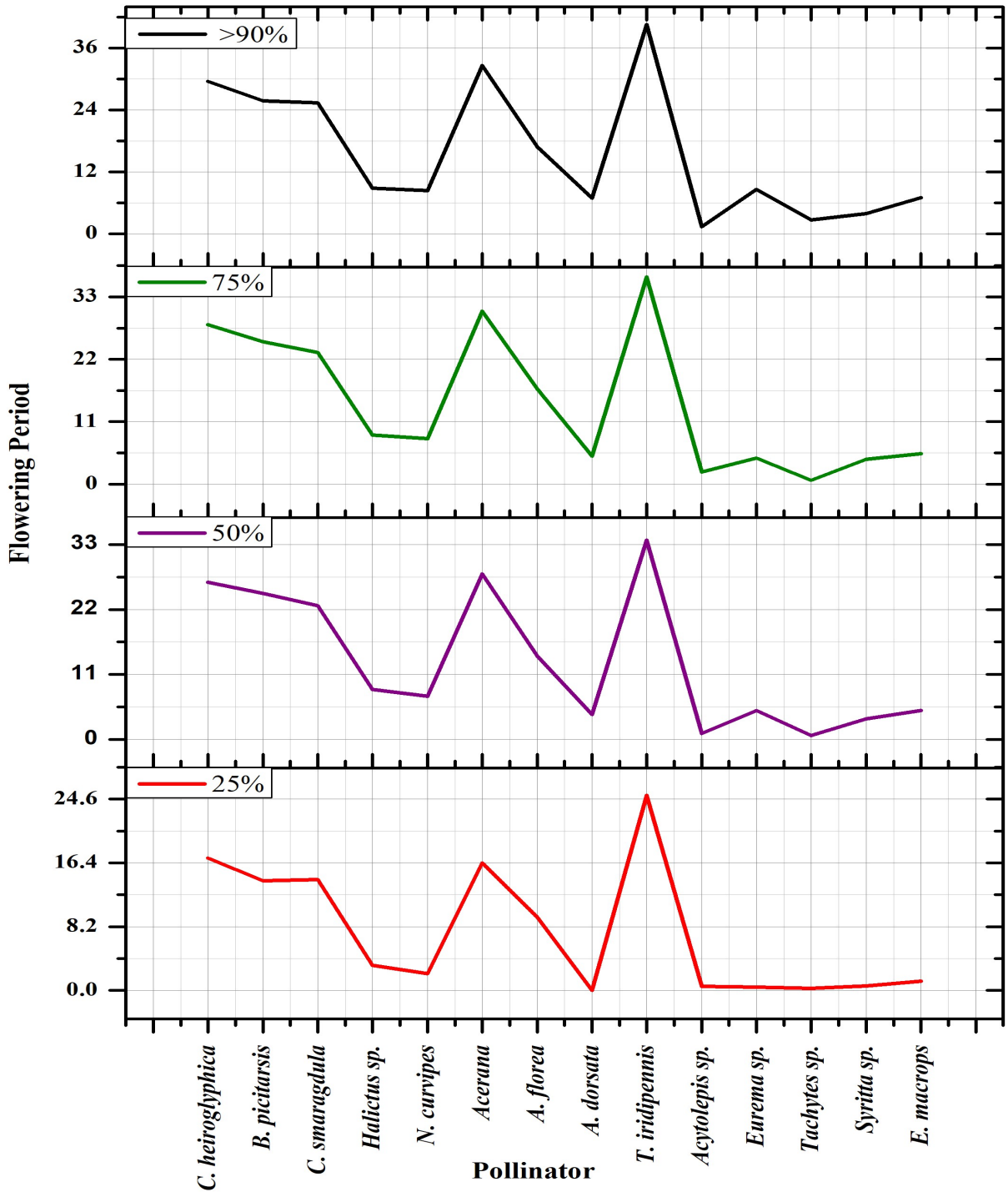


Figure 10. Average number of visit by pollinators in oriental pickling melon ecosystem at different flowering per cent during summer-2019

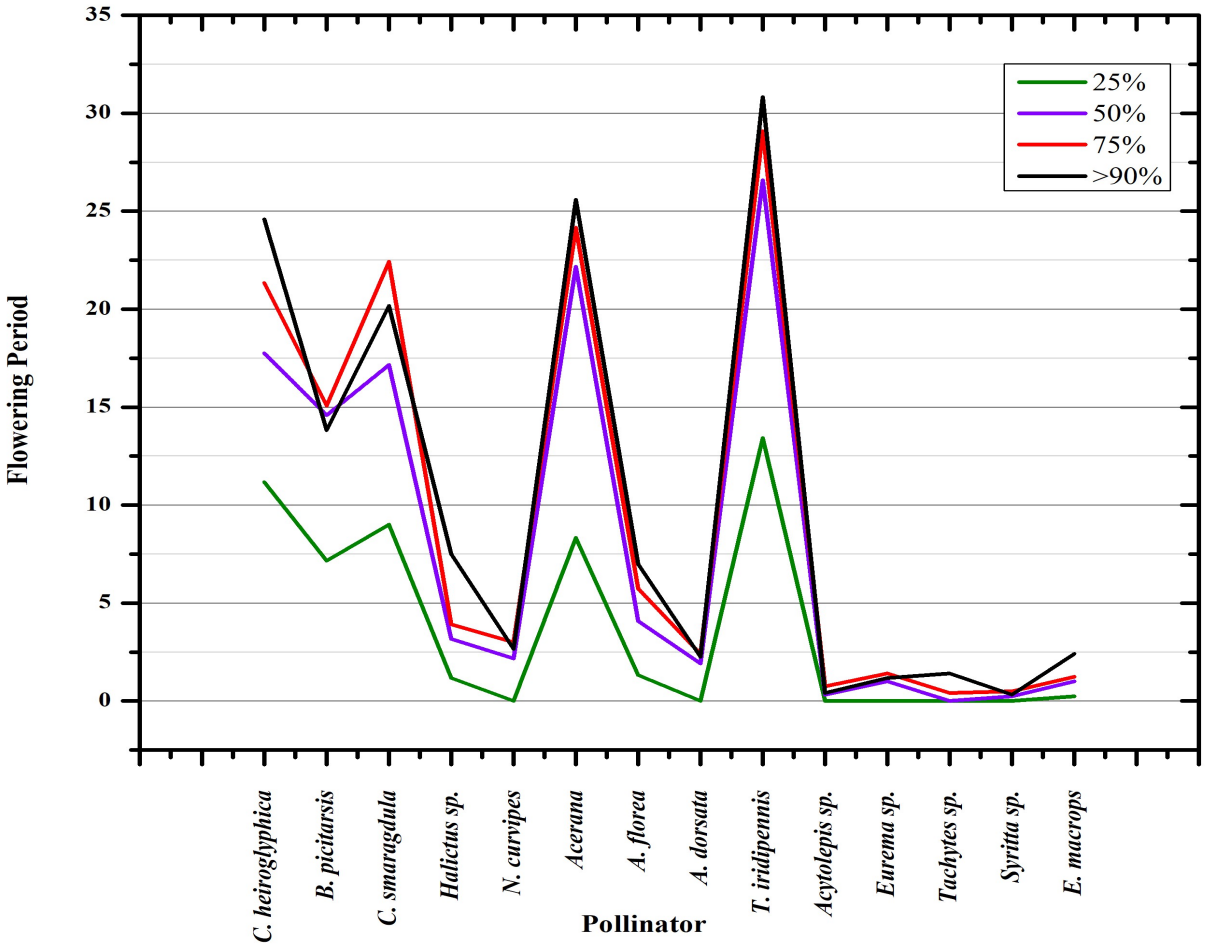


Figure 11. Average number of visit by pollinators in oriental pickling melon ecosystem at different flowering per cent during monsoon-2019

factors (hole sizes, materials and distances) on the per cent occupancy of arthropod nesters.

Hole size diameters of artificial nests significantly influenced the per cent occupancy of both solitary and non-solitary bees. *C. bengalense*, *Dasyproctus* sp., *R. brunneum,* several ant species and spiders were the major non-solitary bee arthropods that occupied the artificial nests during the study. Among them, *Dasyproctus* sp. was confined to construct their nests by occupying the nest hole size of 4 mm whereas, *C. bengalense* preferred the nest hole diameters of 5 and 6 mm. The solitary wasp *R. brunneum* was found occupying in nest hole diameters of 7, 8 and 10 mm. All these hymenopteran non-solitary bee nesters preferred wooden nesting substrate over naturally available tubular hollow plant substrates which were kept at 250 meters away from the field. The solitary bees *i.e*., *B. picitarsis*, *C. smaragdula* and *C. hieroglyphica* were found to occupy nest hole diameters of 2.5, 3 and 3.5 mm and preferred naturally available hollow plant substrates over wooden block nests. All the solitary bee nesters preferred to occupy the artificial nests kept at 250 meters away from the field. The preference and specificity towards the nest hole size diameters could be related to the size of the nesters.

In the present study, species-specific preferences towards the nesting substrates were visible as solitary bees avoided artificial nesting substrates made of wooden blocks, reeds and bamboo, and occupied only in nests made of their natural nesting hosts. Solitary megachild bees *M. disjuncta* and *Megachile* sp. showed species-specific preference towards nesting substrates. *M. disjuncta* preferred to occupy nests made of wooden blocks which were kept 250 meters away from the field whereas, *Megachile* sp. occupied nests made of bamboo and reeds. The major reason behind the preference towards artificial nests kept 250 meters away from the field could be because of fewer human intrusions and safety to brood and progenies as compared to that of the nests kept at distances of 10 m and 100 m wherein human intrusions and safety to nests were low.

In the present study, the occupancy of the major solitary pollen bees species, *B. picitarsis* was recorded 124 days after the installation of the artificial nests, whereas, Vanitha and Raviprasad (2021) reported that the bee species, *B. picitarsis* and *B. mixta* were observed in the artificial nests within 15 days of installation. The

time lag in occupying the artificial nests by the bee species in the present study could be due to factors such as climate during the installation of artificial nests, searching time in finding suitable artificial nests with a proper substrate, hole size and the flowering period of cucurbit crops. In contrast to the study conducted by Vanitha and Raviprasad (2021), *B. picitarsis* were not found to occupy the nests made of wooden blocks and they only preferred nests made of natural host twigs whereas, they reported occupancy of *B. picitarsis* in the artificial nests made of wooden blocks, bamboo sticks, cashew stem sticks, Johnsons grass and Lantana stem sticks. The observations on the preference of nest hole diameters by *B. picitarsis* corroborated with the findings of Vanitha and Raviprasad (2021) in which they also found that *B. picitarsis* preferred nest hole diameters of 2.5, 3 and 3.5 mm.

Udayakumar and Shivalingaswamy (2022) reported the occupancy of three megachilid bees, *Megachile lanata*, *M. laticeps* and *M. disjuncta* in bamboo culms of 15 mm diameter, whereas *M. disjuncta* occupied wooden block nests with diameters 7, 10 and 12 mm diameter in the present study. *Megachile* sp. recorded in the study occupied bamboo culms with diameters of 6 mm and 9.5 mm. Megachilid bees *viz*., *M. concinna* (Alvarez *et al*., 2012) and *M. zaptlana* (Santos *et al*., 2020) also showed a preference for the artificial nest with a cavity diameter of 6 mm.

Ants and spiders were the other arthropod nesters recorded in the present study which occupied nest diameters *viz.*, 8, 9, 9.5, 10, 11, 12, 12.5, 13, 14 and 15 mm. Oliveira *et al.* (2013) found that 19 per cent of plastic nests and 5 per cent of cardboard nests were occupied by ants and spiders implying competition for nesting with bees.

5.3.1. Nesting architecture and nesting biology of two small carpenter bees

The present study observed the nesting architecture and life cycle of two small carpenter bees, *C. hieroglyphica* and *C.smaragdula* which constructed linear nests in pruned dry pithy stems of *C. pulcherrima*, but was rarely found on freshly cut ends of plants. They also found constructing nests in various host plants *viz., Tecoma* sp., *Croton* sp. and *Rosa* spp. According to Udayakumar and Shivalingaswamy (2019) small carpenter bee, *C. binghami,* also found nesting on *C. pulcherrima*, *Adhathoda zeylanica* and *Adenanthera pavonina*. Ali *et al* (2016) reported the nesting activity of *C. smaragdula* in wooden stalks of Ravenna grass (*Saccharaum ravennae* L.). Thus it

was confirmed that the small carpenter bees preferred plants with pithy stems to construct their nests for living. Such plants could be explored for the construction of artificial nests for these small carpenter bees which were identified as the major native bee pollinators of cucurbit ecosystem.

The nests of both species had only one entrance and the entrance diameter did not differ among *C. smaragdula* and *C. hieroglyphica* (two sample t-test, t=0.848, P>0.05). These observations are in line with the study of Yogi and Khan (2014), where they reported that the nest entrance diameters of *Ceratina propinqua* and *Ceratina simillima* had little difference or influence in their nest architecture. Most of the nests were found with adult bees guarding their nests either showing their head or abdomen to ward off natural enemies and thereby protecting their young ones. These observations corroborated with the studies of Kaliaperumal (2019), who reported the presence of adult female bees of *C. hieroglyphica* at the nest entrance to protect their offspring from natural enemies. Most of the nests collected were found with one or two adult bees guarding their nests, whereas some were absent with adult bees in them. These observations were similar to the study by Batra (1976) who reported the presence of old mother bees guarding their nests by buzzing loudly and blocking their nest entrance with the dorsum of their abdomen. Both the species constructed their nests at varied heights (*C. smaragdula*; 61.55±5.34 and *C. hieroglyphica*; 63.42±6.74 (Table 1), with no significant difference in their preference towards the selection of nesting site from the ground (t=⁻0.218, P>0.05). These observations agree with those of Yogi and Khan (2014), who reported that there was no significant difference in height of nests from ground level for the small carpenter bees *viz*., *C. propinqua* and *C. simillima.*

The life cycle of *Ceratina* bees revealed that the eggs are translucent white with cylindrical shapes and convexed ends. Eggs were hatched in 3 to 5 days in both species of bees with no significant difference (Two sample t-test; t=2.861; P>0.05). These results corroborated with the findings of Latha *et al.* (2020) who reported that the small carpenter bee *C. binghami* laid spindle-shaped eggs on pollen balls which took four days for hatching into the first instar larva. Udayakumar and Shivalingaswamy (2019) reported that *C. binghami* took a total larval period of 13.67±1.63 days during development, which was similar to that of the present study

where *C. smaragdula* took 15.51±0.19 days and *C. hieroglyphica* took 15.93±0.27 days for completion of the larval period.

Pupae appeared with the difference in eye colour *viz*., white, pale pink, pink, pale brown, brown and black in accordance to the development period. Pupae with black-coloured eyes showed a difference in body pigmentation at different stages. These observations agree with the results of Kalaiperumal (2019), who reported three consecutive types of pupae based on eye colour in *C. hieroglyphica i.e*., creamy, brown and black.

The total developmental period of both the bee species is not certain in the present study as the adult longevity period might vary based on climate, host plants and various other factors. Ali *et al.* (2016) reported that *C. smaragdula* completed their development within 28 to 32 days in Ravenna grass under laboratory conditions. In the present study, *C. smaragdula* completed its life cycle in an average of 45-54 days, and *C. hieroglyphica* completed its life cycle within 43-53 days.

5.3.2. Nesting architecture and nesting biology of allodapine bee, *B. picitarsis*

The nesting architecture of the allodapine bee, *B. picitarsis* revealed that the average length of nests varied from 5.38±0.38 cm (N=30) in the present study. In *B. mixta* the length of nests was reported to vary from 12 mm to 174 mm (Kaliaperumal *et al*., 2022) whereas in *Braunsapis sauteriella* (Cockerell) it varied from 6.0 mm to 106.0 mm (Shiokawa and Michener, 1977).

The entrance diameter of the nests in *B. picitarsis* was found to be 2.83±0.06 (mm) which was on par with other studies in which, it ranged from 1.1 to 2.5 mm in *B. mixta* (Kaliaperumal *et al*., 2022) and 1.8 to 3.5 mm in *B. sauteriella* (Shiokawa and Michener, 1977). There were no separate pollen provisions for the immature stages of *B. picitarsis* compared to the common stem nesting small carpenter bees (*Ceratina* spp.) in which each egg in the nest was deposited in separate pollen provisions procured by the mother bees (Udayakumar and Shivalingaswamy, 2019).

B. picitarsis nests were thoroughly studied to observe their nesting biology in *C. pulcherrima* twigs. The life cycle of *B. picitarsis* consisted of egg, larva, pupa and adult with a total development period of 56.85±0.84 days. The adult bees laid eggs in groups to the innermost end of the nests and hatched with an average of 4.44±0.14

days under laboratory conditions. But *B. mixta* took an average of six days to hatch with a mean period of 5.38±0.67 days under laboratory conditions (Kaliaperumal *et al*., 2022).

5.4 Study on palynology of solitary pollen bees

The present study revealed the presence of 19 pollen grains belonging to 10 families. Among that, the majority of the pollen was collected from the bee body surface and their nesting sites belonged to the family Fabaceae (27 %) followed by Asteraceae (11 %), Cucurbitaceae (11 %), Lamiaceae (11 %), Porutulacaceae (5 %), Passifloraceae (5 %), Rubiaceae (5 %), Acanthaceae (5 %), Oxalidaceae (5 %) and, Polygonaceae (5 %). Studies conducted by Naim and Phadke (1976) identified that the flora associated with bees could be monitored by the visual observation of bee visits to that particular flora and by their activity of pollen removal from the host flora. In the present study, the foraging bees at the cucurbit ecosystems were monitored thoroughly for observing the associated bee flora. The bees were caught during foraging with pollen on their body and the pollens were observed later. Apart from this, the pollen was also collected from their nesting sites so that, major pollen hosts could be confirmed.

A study conducted by Pradeepa and Belavadi (2018) during 2015-2017 reported 21 pollen taxa associated with leafcutter bees belonged to 8 families. The majority of pollen grains belonged to the family Fabaceae and all the other pollen grains identified were meager in number and belonged to Asteraceae, Bigoniaceae, Lamiaceae, Malvaceae, Covolvulaceae, Rutaceae and Acanthaceae. In the current study conducted to identify the bee flora associated with solitary bees, the majority of the pollen grains belonged to the family Fabaceae, followed by Asteraceae, Cucurbitaceae, Lamiaceae, Porutulacaceae, Passifloraceae, Rubiaceae, Acanthaceae, Oxalidaceae and, Polygonaceae. Thus the similarity in both studies affirms the presence of a majority of bee flora belonging to the family Fabaceae. Cane (2014) found that most of the leafcutter bees exhibited a preference for members of the family Fabaceae because these flowers possessed specific adaptations like keel petals as a strategy to minimize pollen loss by narrowing the spectrum of floral visitors. These findings supported the observations of our present study in which solitary bees also exhibited a special preference for the flora of the family Fabaceae. As the size of

solitary pollen bees was very small, the morphological features might have helped the bees to pollinate the members of the Fabaceae family.

Bhalchandra *et al.* (2014) pointed out that a comprehensive knowledge on bee pasturage was essential to keep the bee keeping potentialities of an area. The present study discovered the natural nesting sites of major solitary pollen bee species which were associated with the selected cucurbitaceous ecosystem such as bitter gourd and oriental pickling melon to promote the availability of their associated floral diversity. The peacock flower tree (*Caesalpinia pulcherrima*) was a major host of small carpenter bees (*C. hieroglyphica* and *C. smaragdula*) and allodapine bee (*B. picitarsis*) as the pollen grains from their nesting sites were abundant as compared to the other pollens. Likewise, the majority of the pollen grains from the nest of megachilid bees, *M. disjuncta* and *Megachile* sp. belonged to the Singapore daisy plant (*Spahgneticola trilobata*). Robertson (1929) observed that the megachilid bees which were coming under solitary bees had shorter foraging ranges and short life cycles with few offsprings, and exhibited higher foraging rates and could trip flowers. In the presented study, it was also observed that solitary bee species identified from the cucurbit ecosystem had shorter life cycle and exhibited shorter foraging range with a few offsprings in their life cycle.

Wcislo and Cane (1996) observed that leaf cutter bees usually collected pollen from a single host to provision a cell in their nest. They also observed that, whenever they found two pollen species in a cell, it could be due to the shortage of pollen of one species in the local area which was compensated by foraging on a taxonomically related flora. Because the nutritional composition of closely related taxa might be similar to each other. These findings were compared to the present study in which, most of the nesting sites of solitary pollen bees were observed with single pollen taxa. It could be because, though solitary bees foraged much flora, they depended on a specific pollen host which could provide all the nutritional requirements to their offspring. Some pollen provisions procured by the adult solitary bees possessed mixed pollen taxa in trace amounts which might have come along with the nectar of the related flora while foraging.

The knowledge of the interaction between the bees and their associated flora is necessary for better management of social and solitary bees. It was observed in the

present study that the availability of pollen and nectar-attracted bees were the major factors affecting their survival, abundance and distribution in an ecosystem.

5.5 Determination of the effect of different plant protection measures on pollination in selected cucurbitaceous crops

Crops such as bitter gourd and oriental pickling melon were raised and the activity of pollinating bees was observed before and after the application of different plant protection measures at >50 per cent flowering of the crops. It was observed that there was a significant reduction in the average number of bee visits starting from the first day after spraying itself. The least average bee visit was recorded in the plants treated with Azadirachtin 300 ppm followed by Imidacloprid 200 SL and Dimethoate 30 % EC in three different seasons *viz*., post-monsoon-2018, summer-2019 and, monsoon-2019.

Tschoeke *et al*. (2019) reported a similar observation from the Neotropical melon fields of Brazil, where they recorded significant reductions in visitations of *Halictus* bees and *A. mellifera* bees which were treated with deltamethrin (alone or mixed with fungicides) and neem-based insecticides. They have also reported that the treatment with fungicides alone did not affect the visitation intensity of any pollinator bees. This was in concordance with the present study where there was little reduction in bee visits to the plants treated with fungicides alone *viz*., Mancozeb 75 WP and Carbendazim 12 WP + Mancozeb 63 WP. But, there was a significant reduction in the bee visit to the plants treated with Azadirachtin 300 ppm and Imidacloprid 200 SL. The visitations to the plants treated with Dimethoate 30 % EC were comparatively higher compared to the plants treated with Azadirachtin 300 ppm and Imidacloprid 200 SL. Reduction in bee visit in the Azadirachtin 300 ppm treated plants was visible in three different seasons of both crops.

Tschoeke *et al*. (2019) also reported that the effective pest control using a pesticide (*i.e.,* neem-based insecticide) not only significantly reduced the visitation intensities of *Halictus* sp. and *A. mellifera*, but also resulted in the lowest productivity of melons. Thus the results reinforce the argument that botanical insecticides should not be exempted from the risk assessment analysis and stresses the importance of conducting complementary assays for botanically based insecticides. Bernardes *et al.* (2018) suggested that neem-based insecticides have been indeed considered excellent

candidates for controlling honey bee pests, but investigations characterizing their field effects on non-*Apis* pollinator bees were still very scarce. Barbosa *et al.* (2015) and Mordue and Blackwell (1993) found that neem-based insecticides have been shown to disrupt the neuroendocrine (altered the functions performed by the prothoracic gland and corpora allatum) and reproductive gland (preventing oogenesis and vitellogenesis by inhibiting cell division and protein synthesis) systems of pollinator bees. Kessler *et al.* (2015) reported that pollinator bees could sense and avoid exposures to chemical compounds (*i.e.,* repelled) which generally reduced the toxic exposure and damage to their colonies, whereas those bees which were not repelled were affected by the toxicity of the chemicals. These studies supported our present findings that, though neem-based insecticides are proposed to apply on crop pests, they could adversely affect the foraging and life cycle of pollinator fauna associated with the crop ecosystems.

5.5.1 Fruit set in bitter gourd and oriental pickling melon in three different seasons

The fruit set in bitter gourd and oriental pickling melon ecosystem was recorded for three different seasons *viz*., post-monsoon-2018, summer-2019 and, monsoon-2019 to assess the impact on fruit yield without pollination. The plants treated with six different plant protection measures were compared to a caged control to assess the difference in yield. It was observed that the plants under caged control yielded a very less number of fruits as compared to the plants in open pollination. The plants treated with fungicides alone yielded more fruits with better fruit weight than the plants under caged control.

A study was conducted by Cane (2005) in raspberries to find the difference in fruit yield with and without pollination by a solitary cavity-nesting bee, *Osmia aglaia* Sandhouse and honey bees. They could find a significant difference in fruit yield and market quality with the pollinating bees in which, they reported that floral visitation by honey bees or *O. aglaia* improved fruit size in terms of druplet counts and fresh fruit weights with bright red colour. Whereas, the plants which were subjected to a no-visit treatment resulted in lighter coloured fruits which were grossly undersized and difficult to pick up and unacceptable for fresh market sales. This findings were

under the present study in which, It was observed that the fruits that yielded under caged plants were of lesser in size than the fruits under open pollination.

Greenleaf and Kremen (2005) recorded native bee pollinators in tomatoes which resulted in a high yield compared to the tomatoes pollinated by managed bee pollinators or self pollination methods. The native bees which were able to sonicate the poricidal anthers could only able to pollinate them while others were found unattractive to them. Thus they suggested that cross pollination with native bee pollinators could enhance the yield of tomato over self pollination. This study could be compared with the present investigation in terms of the ability to pollinate bees which resulted in better yield as compared to that in the caged control.

Murali *et al*. (2021) conducted a similar study in the bitter gourd in which, they reported maximum fruit set in polyhouses with bee pollination with a fruit weight of 255.30 g/fruit whereas, fruit weight under open pollination was 248.60 g/fruit. They also reported that there was no fruit set in bitter gourd under the sleeve caged condition. In contrast to the study, fruit yield was recorded in the bitter gourd which was comparatively very low when compared to the other plants in open-pollinated conditions.

Summary

6. SUMMARY

Studies on "Pollination ecology of solitary pollen bees" were conducted at the Department of Agricultural Entomology, College of Agriculture, during 2018-2021 and the results of the investigation are summarized below.

- Roving surveys were conducted in bitter gourd and oriental pickling melon ecosystems of central districts of Kerala *viz.*, Thrissur, Palakkad, and Ernakulam from 2018-2019 to record the species diversity of all pollinators. A total of 45 insect pollinators were recorded belonging to 11 families of 3 orders. Most abundant insect pollinators belonged to the order Hymenoptera followed by Diptera and Lepidoptera.
- The hymenopterans were the major flower visitors which comprised of 41 species *viz*., *Braunsapis picitarsis* (Cameron), *Braunsapis mixta* (Smith), *Ceratina smaragdula* (F.), *Ceratina binghami* Cockerell, *Ceratina hieroglyphica* Smith, *Ceratina* sp*., Xylocopa ruficornis* Fab., *Xylocopa fenestrate* Fab., *Amegilla zonata* (L.), *Thyreus* sp. 1, *Thyreus* sp. 2, *Apis cerana* Fab., *Apis florea* Fab., *Apis dorsata* Fab., *Tetragonula iridipennis* Smith., *Halictus* sp., *Lasioglossum serenum* (Cameron), *Nomia curvipes* (Fab.), *Hoplonomia elliotti* (Smith), *Gnathonomia thoracica* Smith., *Leuconomia interstitialis* Cameron, *Nomiapis* sp*., Lipotriches* sp., *Coelioxys* sp., *Megachile disjuncta* (Fab.), *Megachile* sp. 1, *Megachile* sp. 2, *Antodynerus punctatipennis* (de Saussure), *Delta pyriforme* (Fabricius), *Eumenes macrops* de Saussure, *Phimenes flavopictus* (Blanchard), *Rhynchium brunneum* (Fabricius), *Ropalidia brevita* Das & Gupta, *Phalerimeris phalerata turneri* (Betrem), *Scolia affinis* (Guerin), *Scolia cyanipennis* Fabricius, *Chalybion bengalense* (Dahlbom), *Sphex argentatus* Fabricius*, Sphex sericeus* (Fabricius), *Chrysis* sp. and *Tachytes* sp.
- *T. iridipennis* was found to be the predominant species in the sweep net collection followed by *A. cerana*, *A. florea, C. hieroglyphica, C. smaragdula,* and *B. picitarsis*, in all the three districts.
- The diversity indices of pollinators revealed that the Thrissur district has a higher number of taxa (33) followed by Palakkad (29) and Ernakulam (17) districts. Simpson's diversity index (1-D) and Shannon index (*H'*) values were higher for the Thrissur district which was followed by Palakkad and

Ernakulam districts. Brillouin index (H_B) was found the highest in the Thrissur district whereas the Berger-Parker index was found higher in the Ernakulam district. Menhinick's index was higher in the Palakkad district whereas, Margalef's index was high in the Thrissur district. Pielou's evenness index (J) was found to be high in the Thrissur district followed by the Ernakulam and Palakkad districts.

- The relative abundance of solitary pollen bees varied in each district. The diversity study revealed that the Palakkad district has a high number of taxa (19) followed by Thrissur (13) and Ernakulam (8) districts. The Simpson's diversity index (1-D) values showed that the Thrissur and Palakkad districts were more species-rich and uniformly distributed when compared to the Ernakulam district. Shannon diversity index (H') was high in the Palakkad district. Though the Brillouin index of the Thrissur and Palakkad districts were almost similar, species abundance was higher in the Thrissur district related to the sample size. The Berger-Parker index was found higher in the Ernakulam district whereas Menhinick's index and Margalef's index were high in the Palakkad district. Pielou's evenness index showed that pollinator species were more uniformly distributed in the Ernakulam district.
- Molecular characterization of 15 solitary pollen bee samples was done to confirm the species' identity. The DNA sequences were combined to get the contigs and uploaded to the NCBI GenBank database and accession numbers were generated. The same sequences were then uploaded to BOLD systems and barcodes and BIN numbers were also generated.
- In bitter gourd, the mean number of solitary pollen bees per square meter area (N=100 days) was the highest during the hour, 9.00 AM to 10.00 AM during post-monsoon 2018 (446±40.90), summer-2019 (467.20±33.50) and monsoon-2019 (353±22.60) seasons. Whereas, the least mean number of solitary pollen bees were recorded during the hour 6.00 AM to 7.00 AM in all three seasons *viz*., post-monsoon 2018 (206.40±29.70), summer-2019 (205.80±27.50) and monsoon-2019 (124.20±12.90).
- In oriental pickling melon, the mean number of solitary pollen bees per square meter area (N=100 days) was the highest during the hour, 9.00 AM to 10.00 AM during post-monsoon 2018 (369.40±16.40) and monsoon 2019

(343.80±27.60) seasons. However, the highest mean number of solitary pollen bees per square meter area in summer-2019 was during the hour 10.00 AM to 11.00 AM (386.60±20.20). The least mean number of solitary pollen bees were recorded during the hour 11.00 AM to 12.00 PM in all three seasons *viz*., post-monsoon-2018 (177.60±14.10), summer-2019 (168.40±14.30) and, monsoon-2019 (186.80±13.60).

- The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in bitter gourd ecosystem showed that, *T. iridipennis* was the most abundant pollinator followed by *A. cerana*, *B. picitarsis* and, *C. hieroglyphica* in all the three seasons. *B. picitarsis* was the most abundant pollinator among the solitary pollen bees observed under bitter gourd ecosystem.
- The visual counts on all flower visitors with respect to their abundance at 100 per cent flowering stage in oriental pickling melon ecosystem showed that, *T. iridipennis* was the most abundant pollinator followed by *A. cerana* in all the three seasons. *B. picitarsis* and, *C. hieroglyphica* in all the three seasons.
- The allodapine bee, *B. picitarsis* (14.79 %) was the most abundant pollinator among the solitary bees during post-monsoon-2018, followed by *C. hieroglyphica* (14.20 %) and, *C. smaragdula* (5.19 %). Whereas, summer-2019 season was observed with *C. hieroglyphica* as the most abundant pollinator (13.52), followed by *B. picitarsis* (11.80 %) and, *C. smaragdula* (11.61 %). *C. hieroglyphica* was the most abundant with a per cent abundance of 17.53 in monsoon-2019, followed by *C. smaragdula* (14.38 %) and, *B. picitarsis* (9.86 %).
- The stingless bee, *T. iridipennis* was found as the pollinator species to give maximum number of visit to bitter gourd flowers in three seasons at four different flowering percentages. *B. picitarsis* was the solitary bee species to give maximum flower visit to bitter gourd flowers in three different seasons at four different flowering percentages.
- The small carpenter bee, *C. hieroglyphica*, *B. picitarsis* and *C. smaragdula* were the solitary bee species to give maximum flower visit to oriental pickling melon flowers in three different seasons at four different flowering percentages, whereas *T. iridipennis* was found as the pollinator species to give

maximum number of visit to bitter gourd flowers in three seasons at four different flowering percentages.

- Nesting preferences based on the per cent occupancy of nests revealed that, hole size diameters, nesting substrate and distances from the field of artificial nests significantly influenced the domiciliation of both solitary and non-solitary bees.
- The solitary bees *i.e*., *B. picitarsis*, *C. smaragdula* and *C. hieroglyphica* were found to occupy nest hole diameters of 2.5, 3 and 3.5 mm and preferred naturally available hollow plant substrates over wooden block nests. All the solitary bee nesters preferred to occupy the artificial nests kept at 250 meters away from the field.
- The nesting architecture of two small carpenter bees, *C. smaragdula* and *C. hieroglyphica* showed that they constructed linear nests at soft pithy region of stems with a maximum of 12 cm depth and individual cells ranged 6 to 10 mm in length which were separated with partitions of 2 to 4 mm. The younger cells of bees were near to the entrance of nests, whereas the mature cells were at the innermost side. The life cycle consisted of egg, larva, pupa and adult stages and *C. smaragdula* and *C. hieroglyphica* took 49.15±0.40 and 43.19±0.58 days to complete their total life cycle under laboratory conditions.
- The nesting architecture of allodapine bee, *B. picitarsis* revealed that the bees preferred nesting sites with an entrance diameter of 2.83±0.06 mm with a nest length of 5.38±0.30 cm. The life cycle consisted of egg, larva, pupa and adult with a total development period of 56.85±0.84 days under laboratory conditions. Pupa showed difference in their eye colour and body pigmentation during their developmental period.
- A total of nineteen pollen grains belonging to 10 families were identified during the palynological studies. Among that, majority of the pollen was collected from the bee body surface and their nesting sites belonged to the Family Fabaceae (27 %) followed by Asteraceae (11 %), Cucurbitaceae (11 %), Lamiaceae (11 %), Portulacaceae (5 %), Passifloraceae (5 %), Rubiaceae (5 %), Acanthaceae (5 %), Oxalidaceae (5 %) and, Polygonaceae (5 %).
- There were significant reduction in the average number of bee visit to crop plants starting from the frist day after spraying of the selected plant protection

measures. The least average bee visit was recorded in the plants treated with Azadirachtin 300 ppm followed by Imidacloprid 200 SL and Dimethoate 30 % EC in three different seasons *viz*., post-monsoon-2018, summer-2019 and, monsoon-2019. However there were little reduction in bee visit to the plants treated with fungicides alone *viz*., Mancozeb 75 WP and Carbendazim 12 WP + Mancozeb 63 WP.

- The plants under caged control yielded very less number of fruits as compared to the plants in open pollination. The plants treated with fungicides did not affect bee visit significantly and those plants yielded more number of fruits with better fruit weight than the plants under caged control.

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Appendices

Appendix-1

Abbreviations and units used

Abbreviations

MSL: Mean Sea Level

AM: Ante Meridiem

PM: Post Meridiem

PAST: Paleontological Statistics Software Package for Education and Data Analysis

D_{Mg}: Margalef's index

D_{Mn}: Menhinick's index

H′: Shannon-Weiner diversity index

D: Simpson diversity index

J′: Pielou's evenness index

DNA: Deoxyribo Nucleic Acid

RNA: Ribo Nucleic Acid

OD: Optical Density

mtCOI: Mitochondrial Cytochrome Oxidase I

PCR: Polymerase Chain Reaction

CAP3: Contig Assembly Program 3

NCBI: National Center for Biotechnology Information

BLASTn: Basic Local Alignment Search Tool for nucleotides).

BOLD: Barcode of Life Data System

SPSS: Statistical Package for the Social Sciences

GRAPES: General R-shiny based Analysis Platform Empowered by Statistics

KAU: Kerala Agricultural University

BCCP: Biological Control of Crop Pests

KVASU: Kerala Veterinary and Animal Sciences University

PalDat: Palynological Database

Units

ºC: degree Celsius

m: meter

mm: millimeter

%: per cent

µl: microliter

sec: seconds

min: minutes

rpm: revolutions per minute

h: hour

nm: nanometer

ng: nanogram

RH: relative humidity

ml: milliliter

µm: micrometer

a.i.: active ingredient

Appendix- II

Weather data Parameters during the period of study (2018-2021)

Mean Maximum Temperature (^{o}C)				
Months	**2018**	**2019**	**2020**	**2021**
January	33.5	32.9	34.1	32.3
February	35.7	35.3	35.5	34.6
March	36.7	36.8	36.4	36.8
April	36.1	36.1	36.4	34.9
May	33.2	34.6	35.0	32.7
June	29.8	32.2	31.1	31.2
July	29.6	30.4	30.5	29.8
August	29.2	29.5	30.2	30.2
September	32.2	31.2	30.0	30.7
October	32.8	32.4	31.0	31.3
November	32.7	32.9	33.0	31.0
December	33.0	32.3	32.0	32.5
Mean	32.8	33.1	32.9	32.3

Mean Minimum Temperature (^{o}C)				
Months	**2018**	**2019**	**2020**	**2021**
January	20.9	20.4	22.4	21.3
February	22.5	23.4	23.2	21.6
March	24.0	24.8	24.4	23.0
April	24.8	25.5	24.7	23.6
May	22.6	24.9	25.2	22.9
June	23.2	23.5	23.7	23.7
July	22.5	22.8	23.2	23.5
August	22.2	21.7	23.1	23.4
September	22.5	22.0	22.4	23.9
October	22.9	21.4	21.5	23.6
November	23.3	21.7	22.0	23.4
December	22.5	22.1	21.9	23.3
Mean	22.8	22.8	23.1	23.1

Weather data Parameters during the period of study (2018-2021) (contd.)

Mean Relative Humidity (%)				
Months	**2018**	**2019**	**2020**	**2021**
January	53	55	61	64
February	47	59	54	54
March	59	65	65	59
April	69	70	71	73
May	79	74	77	83
June	89	83	85	84
July	88	85	87	87
August	87	89	87	86
September	75	85	88	83
October	76	80	82	86
November	68	71	70	81
December	63	63	65	67
Mean	71.1	73.3	74.3	75.6

Mean Relative Humidity Morning (%)				
Months	**2018**	**2019**	**2020**	**2021**
January	68	71	78	78
February	63	77	71	70
March	79	85	85	84
April	85	86	86	89
May	91	89	90	94
June	95	93	94	94
July	96	95	96	96
August	96	96	96	96
September	91	95	96	96
October	90	91	95	96
November	82	83	84	91
December	78	73	75	80
Mean	84.5	86.2	87.2	88.6

Weather data Parameters during the period of study (2018-2021) (contd.)

Mean Relative Humidity Evening (%)				
Months	**2018**	**2019**	**2020**	**2021**
January	37	38	43	50
February	30	41	37	38
March	39	45	46	34
April	54	54	55	58
May	66	59	63	73
June	83	73	75	74
July	80	76	78	77
August	78	82	77	76
September	60	75	80	71
October	62	68	69	77
November	54	60	57	71
December	47	52	55	55
Mean	57.5	60.3	61.3	62.8

Rainfall (mm)				
Months	**2018**	**2019**	**2020**	**2021**
January	0.0	0.0	0.0	45.7
February	5.2	0.0	0.0	0.0
March	33.2	0.0	33.4	31.8
April	28.9	76.4	44.7	72.4
May	483.6	48.8	59.6	550.5
June	730.0	324.4	427.2	473.0
July	793.2	654.4	563.0	626.9
August	928.0	977.5	607.7	409.1
September	29.0	419.0	587.6	291.7
October	393.0	418.4	310.3	593.2
November	66.6	205.4	56.1	364.2
December	0.0	4.4	7.7	19.2
Mean	290.8	260.7	224.7	289.8
Total	3490.7	3128.7	2697.3	3477.7

Weather data Parameters during the period of study (2018-2021) (contd.)

Rainy Days				
Months	**2018**	**2019**	**2020**	**2021**
January	0	0	0	1
February	1	0	0	0
March	2	0	2	1
April	2	3	4	4
May	14	4	5	16
June	23	15	20	21
July	22	21	21	22
August	21	24	17	22
September	1	19	21	14
October	13	16	12	17
November	5	5	2	13
December	0	1	1	1
Total	104	108	105	132

Mean Evaporation (mm/day)				
Months	**2018**	**2019**	**2020**	**2021**
January	4.4	4.7	4.9	4.3
February	5.6	5.1	5.9	5.5
March	5.0	4.8	4.8	5.3
April	4.3	4.7	4.6	3.7
May	3.3	4.0	3.7	2.7
June	2.2	2.8	2.3	2.7
July	2.6	2.4	2.5	2.1
August	2.3	1.9	2.5	2.2
September	3.3	2.5	2.1	2.6
October	3.0	2.7	2.4	2.0
November	3.4	3.4	3.6	2.1
December	3.5	4.5	4.4	4.0
Mean	3.5	3.6	3.6	3.2

Weather data Parameters during the period of study (2018-2021) (contd.)

Total Evaporation (mm)				
Months	**2018**	**2019**	**2020**	**2021**
January	135.5	144.8	151.0	132.4
February	157.9	143.4	171.0	155.1
March	155.2	148.8	148.1	164.4
April	127.8	142.1	136.6	112.2
May	102.9	122.5	115.2	82.8
June	65.7	84.4	69.9	77.0
July	79.6	73.8	76.7	64.6
August	70.7	59.1	78.3	69.2
September	99.6	75.2	62.3	76.9
October	94.4	84.0	75.5	61.7
November	102.3	101.5	107.4	62.0
December	109.5	140.7	135.2	123.6
Mean	108.4	110.0	110.6	98.4
Total	1301.1	1320.3	1327.2	1181.9

Total Sunshine Hours (h)				
Months	**2018**	**2019**	**2020**	**2021**
January	254.1	261.4	290.2	206.1
February	265.1	244.4	275.8	256.4
March	247.6	265.4	263.9	267.9
April	217.7	240.7	241.5	188.9
May	149.0	211.0	190.0	138.7
June	57.2	111.7	74.0	129.9
July	58.0	81.6	86.9	75.3
August	68.4	45.9	94.9	78.2
September	216.2	98.3	70.8	118.5
October	176.0	170.2	170.0	109.4
November	207.5	224.9	198.5	73.4
December	215.7	208.8	193.9	254.2
Mean	177.7	180.3	179.2	158.1
Total	2132.5	2164.3	2150.4	1896.9

Weather data Parameters during the period of study (2018-2021) (contd.)

Mean Sunshine hours (h/day)				
Months	**2018**	**2019**	**2020**	**2021**
January	8.2	8.4	9.4	6.6
February	9.5	8.7	9.5	9.2
March	8.0	8.6	8.5	8.6
April	7.3	8	8.1	6.3
May	4.8	6.8	6.1	4.5
June	1.7	3.7	2.5	4.3
July	1.9	2.6	2.8	2.4
August	2.2	1.5	3.1	2.5
September	7.2	3.3	2.4	4.0
October	5.7	5.5	5.5	3.5
November	6.9	7.5	6.6	2.4
December	7.0	6.7	6.3	8.2
Mean	5.8	5.9	5.9	5.2

Mean Wind Speed (km/h)				
Months	**2018**	**2019**	**2020**	**2021**
January	5.4	6.5	5.9	5.9
February	5.7	5.4	5.3	5.9
March	3.3	2.9	2.8	3.0
April	2.0	2.3	2.5	2.0
May	1.8	2.0	2.2	1.5
June	1.5	1.7	1.3	1.5
July	1.7	1.7	1.1	1.4
August	1.8	1.5	1.8	1.6
September	1.7	1.4	1.5	1.7
October	2.0	1.8	1.5	1.5
November	4.3	4	4.4	2.1
December	4.7	8.7	6.7	5.5
Mean	2.9	3.3	3.1	2.8

POLLINATION ECOLOGY OF SOLITARY POLLEN BEES

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ABSTRACT

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Pollination ecology of solitary pollen bees

Abstract

Bees are often considered to be effective pollinators in both agricultural and natural ecosystems. The recent declines in feral and domesticated social bee populations have raised serious concerns about their impact on the productivity of ecosystems and this urges the need to explore potential alternate bee pollinators for the future. There are 20,000 known species of bees in seven recognised biological families and more than 90 per cent of them are solitary. Unlike social bees, solitary pollen bees establish and provision nests on their own with no assistance from other individuals. Solitary bees play an immensely important role in the pollination of crop plants as well as wild plants. In India, little attempt has been made in documenting solitary bee species. This study was therefore undertaken to document the diversity of solitary pollen bees in selected cucurbitaceous ecosystems with a major emphasis on determining their peak foraging time, nesting preferences, palynology, and the effect of plant protection practices on pollination.

Roving surveys were conducted to document the pollinator diversity in bitter gourd and oriental pickling melon ecosystems of central districts of Kerala *viz.*, Thrissur, Palakkad, and Ernakulam from 2018 to 2019. A total of 45 insect pollinator species were recorded from 11 families of three insect orders. Morphological characterisation of insect pollinators revealed that 23 species were solitary pollen bees belonging to the families, Apidae, Halictidae and Megachilidae. Molecular characterisation and DNA barcoding of solitary pollen bees under the same genera were done to confirm the species' identity.

The peak foraging time of solitary pollen bees was determined by counting the total number of bees visiting the gourd flowers in a square meter area for 100 days. In the bitter gourd ecosystem, the peak bee visit was between 9.00 am and 10.00 am with an average number of 446 ± 40.90, 467.20 ± 33.50 and 353 ± 22.60 bees/m^2 in post-monsoon-2018, summer-2019 and monsoon-2019 seasons, respectively. Whereas, the least number of solitary bees were observed between 6.00 am and 7.00 am with an average of 206.40 ± 29.70, 205.80 ± 27.50 and 124.20 ± 12.90 bees/ m^2 in post-monsoon-2018, summer-2019 and monsoon-2019 seasons, respectively.

In the oriental pickling melon ecosystem, the peak foraging time of solitary pollen bees was recorded between 9.00 am and 10.00 am with an average of 369.40 ± 16.40 and 343.80 ± 27.60 bees/m^2 in post-monsoon-2018 and monsoon-2019 seasons. Whereas, in summer-2019, it was between 10.00 am and 11.00 am with an average of 386.60 ± 20.20 bees/m^2. The least mean number of solitary pollen bees in the oriental pickling melon ecosystem were observed between 11.00 am and 12.00 pm with an average of 177.60 ± 14.10, 168.40 ± 14.30 and 186.80 ± 13.60 in post-monsoon-2018, summer-2019 and monsoon-2019, respectively. The visual counts on all flower visitors to their abundance at 100 per cent flowering stage in bitter gourd and oriental pickling melon ecosystems showed that *Tetragonula iridipennis* Smith was the most abundant pollinator followed by *Apis cerana* Fab., *Braunsapis picitarsis* (Cameron) and *Ceratina hieroglyphica* Smith in all the three seasons. The allodapine bee, *B. picitarsis* and the small carpenter bee, *C. hieroglyphica* were the solitary pollen bee species that made the maximum number of visits to bitter gourd and oriental pickling melon flowers respectively in all recorded seasons at 25, 50, 75 and >90 per cent flowering stages.

The assessment of per cent occupancy of the artificial nests revealed that the solitary pollen bees other than the megachild bees *viz*., *B. picitarsis*, *C. hieroglyphica*, and *C. smaragdula* (F.) preferred natural host nests to wooden nests with specific hole sizes *viz*., 2.5, 3.0 and 3.5 mm. The solitary megachilid bees preferred wooden as well as the natural host nests with hole sizes of 6, 7, 9, 10 and 12 mm. All the solitary bees were found to occupy the nesting blocks kept 250 meters from the field area.

The pollens collected from the body surface and nesting sites of solitary pollen bees revealed nineteen pollen taxa belonging to 10 families which were identified by light microscopy and scanning electron microscopy. Among those, the majority of pollen taxa belonged to the family Fabaceae (26 %) followed by Asteraceae (16 %), Cucurbitaceae (16 %), Lamiaceae (11 %), Portulacaceae (5 %), Passifloraceae (5 %), Rubiaceae (5 %), Acanthaceae (5 %), Oxalidaceae (5 %) and Polygonaceae (5 %).

There was a significant reduction in the average number of bee visits to crop plants *i.e*., bitter gourd and oriental pickling melon with the least average bee visit recorded in the plants treated with azadirachtin (300 ppm) followed by imidacloprid (200 SL) and dimethoate (30 % EC) in three different seasons *i.e*., post-monsoon-

2018, summer-2019 and monsoon-2019. However, there was little reduction in bee visits to the plants treated with fungicides alone *i.e.*, mancozeb (75 WP) and carbendazim (12 WP) + mancozeb (63 WP). The plants under caged control yielded a significantly fewer number of fruits as compared to the plants in open pollination. The plants treated with fungicides alone yielded more fruits with better weight than those under caged control.

In conclusion, the present study documented major solitary pollen bees in the cucurbitaceous ecosystem. The results reinforce the argument that botanical insecticides should not be exempted from the risk assessment analysis and stresses the importance of conducting complementary assays for botanically based insecticides. The preference of major solitary pollen bees for the artificial nesting sites recommends the provision of solitary bee flora as hedge plants in the field so that maximum productivity of the crops can be ensured.