POLLINATION EFFICIENCY OF STINGLESS BEE, *Tetragonula iridipennis* (Smith) IN CUCUMBER (*Cucumis sativus* L.) UNDER PROTECTED CULTIVATION

by

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(2018-11-170)

THESIS

Submitted in partial fulfilment of the requirements for the degree of

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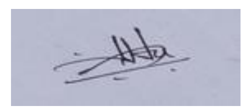
2020

DECLARATION

I, hereby declare that this thesis entitled **"POLLINATION EFFICIENCY OF STINGLESS BEE, *Tetragonula iridipennis* (Smith) IN CUCUMBER (*Cucumis sativus* L.) UNDER PROTECTED CULTIVATION"** 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 or other similar title, of any other University or Society.



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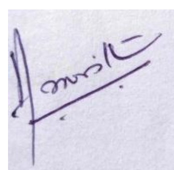

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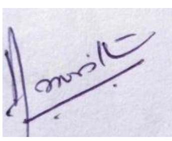

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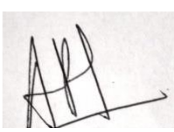

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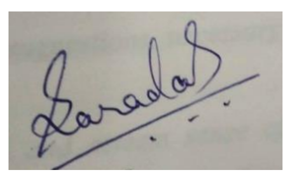

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Lintu V. V.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

et al.	And other co workers
Cm	Centimeter
R	Correlation coefficient
CD	Critical difference
€	Currency symbol of euro
DAS	Days after sowing
°C	Degree Celsius
G	Gram
Ha	Hactare
H	Hour
Kg	Kilogram
μL	Microlitre
mL	Milliliter
Mm	Millimeter
Min	Minute

MAS	Months after sowing
R^2	Multiple correlation coefficient
viz.,	Namely
NS	Non significant
no.	Number
%	Per cent
$fruit^{-1}$	Per fruit
male $flower^{-1}$	Per male flower
min^{-1}	Per minute
m^{-2}	Per square meter
sec.	Second
sp. or spp.	Species (Singular and plural)
UV	Ultra violet

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Introduction

1. INTRODUCTION

Pollination is one of the crucial events in the life cycle of gymnosperms and angiosperms whereby pollen is transferred from the male to the female part of the flower. This plays a vital role in sustainable agriculture, since production and propagation of flowering crops relies on either self or cross pollination methods. Among the various methods of cross pollination, pollination by animals is an important ecosystem service because 35 per cent of worldwide crop - based food production is benefitted from animal-mediated pollination. Bees (Hymenoptera: Apiformes), are the primary pollinators for most of the crops requiring animal pollination (Free, 1993; Thapa, 2006; Klein *et al.*, 2007; Bareke and Addi, 2019). They are the foremost efficient and reliable pollinators in increasing crop productivity throughout the globe because of their method of communication and morphological adaptations to flower parts.

The worldwide economic value of pollination service provided by insect pollinators, mainly bees, is €153 billion, which represents 9.50 per cent of the total value of the world agricultural food production. Their disappearance would translate into a consumer surplus loss estimated between €190 and €310 billion (Gallai *et al.,* 2009).

Even though our agricultural fields are blessed with a plethora of pollinators, augmented pollination with domesticated bees are largely exploited in the open field conditions (Premila *et al*., 2014). Evolution in agricultural practices, demand the rise in production and productivity without expanding area. One of the improved production technologies to cope up with adverse climatic condition in many parts of the world is protected cultivation. Greenhouse crop production is now a growing reality throughout the planet with an estimate of 405,000 ha of greenhouses spread over all the continents. There are more than 55 countries now in the world where protected cultivation of crops is undertaken on an advert scale. The total greenhouse area in India is also increasing with a current estimate of 30,000 ha (Reddy, 2016). The expansion of protected cultivation in world agriculture has driven crops traditionally cultivated in open field to greenhouse.

Cucumis sativus L. is one of the widely cultivated warm season vegetable crops mainly grown in tropical and sub-tropical regions. Since the crop prefers slightly low temperature, it is compatible for polyhouse cultivation. Cucumbers, being a monoecious crop, male and female flowers are borne separately and they need the assistance of external agents for successful pollination (Free, 1993). Even though hybrids that are parthenocarpic are opted for enclosed condition, farmers prefer highly cross pollinated popular varieties which require external agents for their successful pollination. Cucumber flowers are known to be visited by a number of pollinators, even then decrease of seed and fruit set also occur due to the insufficiency of insect pollinators (Srikanth, 2012). The lack of pollinators reduces the yield by 75 per cent compared to the potential maximum (Motzke *et al.*, 2015).

Before 1990s, plant growth regulators which are also called as hormone spray were frequently used for greenhouse crop pollination (Gosterit and Gurel, 2018). Pollination with the aid of vibrators and hand pollination are also common. These methods are tedious, time consuming and labour intensive and are not economical.

Though, live pollinators like hover flies (Diptera: Syrphidae) can be managed under protected condition (Jarlan *et al*., 1997), bees are most efficient, reliable and cheap alternatives. Among 16,000 known species of bees (Michener, 2000), a few species are used for commercial pollination. Managed bee pollinators that are good candidates as commercial pollinators include honey bees like European bee (*Apis mellifera* Linnaeus), Indian bee (*Apis cerana indica* Fabricius), stingless bee (Meliponini), bumble bee (*Bombus* spp.) and a few solitary bee species like *Xylocopa* sp. (Hogendoorn, *et al*., 2000), *Amegilla* spp. (Bell *et al*., 2006; Hogendoorn *et al*., 2006) and *Anthophora* sp. (Adhikari and Miyanaga, 2015).

Stingless bees play a crucial ecological role as pollinators of many wild plant species and appear as good candidates for future alternatives in commercial pollination (Amano, 2004). The number of crops reported to be effectively pollinated by stingless bees exceeds 18. Eleven stingless bee species across six genera have been found to forage effectively under enclosed conditions, indicating the potential of stingless bees as pollinators of greenhouse crop (Slaa *et al.*, 2006).

It is possible to keep individual stingless bee hives for long periods. The colonies are naturally long-lived unlike bumble bees that may perish after reproduction. They lack a functional sting, which makes them especially suitable for pollination of crops that are cultivated in inhabited areas and in enclosures such as cages and greenhouses. Under adequate climatological conditions they forage year-round. This makes them especially suitable for offseason production of crops in green houses (Amano *et al*., 2000). Many stingless bee species have proven to forage well in enclosed areas compared to highly defensive African-derived *A. mellifera* or non-native bumble bees (*Bombus* spp.) (Cauich *et al*., 2004; Bomfim *et al*., 2016).

Most species of stingless bees have a foraging range smaller than that of the honey bee (Wille, 1983). Unlike other bees they can also forage even if there is lack of ultraviolet rays inside green house where the roof is made of UV proof materials (Tezuka and Maeta, 1993). These features can ensure foraging efficiency in confined spaces. Furthermore, they suffer from fewer diseases, pests and parasites than the honey bee which simplifies colony management (Slaa *et al.*, 2006). However, studies on the pollination aspects under protected cultivation using stingless bee for yield enhancement is much scanty in India. This study entitled "Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) in cucumber (*Cucumis sativus* L.) under protected cultivation" was carried out with following objective:

- To evaluate the effect of pollination by stingless bee, *Tetragonula iridipennis* (Smith) on fruit set, yield and quality of salad cucumber under protected cultivation

Review of Literature

2. REVIEW OF LITERATURE

The literature relevant to foraging behaviour of stingless bees in polyhouse, floral biology of cucumber and the yield enhancement by bee pollination compared to hand pollination are reviewed under the following headings.

2.1 FORAGING BEHAVIOUR OF STINGLESS BEES IN POLYHOUSE

Pollination is the most important contribution by bees to human community. Bees adopt their foraging behaviour in accordance with floral availability, biology of crop and the internal needs of the colonies. Also there is a flexible response to prevailing weather parameters (Kleinert-Giovannini and Imperatriz-Fonseca, 1986). Efficient pollinator must acquire required amount of pollen and deposit it, in a viable state, on the receptive stigma of flowers (Heard, 1994).

2.1.1 Mode of Alighting of Individual Foragers Visiting the Bloom

The foragers of stingless bees adopt different methods to approach flowers, to access the pollen and nectar rewards. In these processes, the bees involuntarily contact the reproductive parts of the flowers. According to Barrows (1976) foragers of *Trigona fulviventris* Guerin were nectar-foraging-perforating robbers of flowers of *Lantana camara* L.. In macadamia flowers, some of the stingless bees (*Trigona carbonaria* Smith) foragers (pollen collectors), upon alighting on the raceme, climbed to the top of the styles and make direct contact with stigma while others (nectar collectors) remained at the base of the flower and, never reach the style (Heard, 1994). Pratap (2010) reported that the bees landing directly on the top of reproductive part of a flower (top-workers) carry more pollen on their body than those lands on the petals (side-worker).

Bomfim *et al.* (2014) observed that the individuals of *Scaptotrigona* sp. either land on the petals of staminate and pistillate flowers of mini watermelon and then reach nectary at the bottom of corolla or they land directly on the reproductive parts of the flowers (anthers and stigma in staminate pistillate flowers, respectively), where they proceeded towards the nectary. Some foragers perform both type of behaviour in the same flower during the same visit.

2.1.2 Initiation and Cessation Time of Foraging Activity

The foraging activity of stingless bees *Trigona minangkabau* (Hoshiba and Imai) was uniform throughout the daytime at fine weather conditions in the original distribution area, Sumatra (Inoue *et al*., 1985), while it is limited to the period from 0900 to 1400 h even at fine weather conditions when introduced to the greenhouse of Matsue, Japan (Kakutani *et al*., 1993).

Nicodemo *et al*. (2013) recorded the visit of *Nannotrigona testaceicornis* (Lepeletier) bees to parthenocarpic cucumber flowers was from 0700 to 1600 h and the visit become more frequent between 1000 and 1200 h. Tej *et al*. (2017) reported that there is a temporal variation in foraging activity of *T. iridipennis* in two different greenhouses with cucumber cultivation. The foraging period was recorded from 0700 to 1800 h in one greenhouse at TNAU orchard (11.01^{o}N 76.93^{o}E, 427m above MSL) and from 0600 to 1800 h in another greenhouse at Srivilliputhur (9°30N 77°38, 137m above MSL).

While evaluating neotropical stingless bee *Melipona quadrifasciata* Lepeletier for pollinating greenhouse grown hybrid tomatoes, del Sarto *et al*. (2005) reported that the bees foraged from 0800 to 1100 h and most foraging activity (62.50 %) occurred from 0900 to 1100 h.

According to Roselino *et al.* (2009) the foraging activity of both bee species, *Scaptotrigona* aff. *depilis* Moure and *N. testaceicornis*, overlap with the time of anthesis of strawberry. Both the bees visited strawberry flowers from 0900 to 1600 h. The former in one greenhouse had the peak activity between 1300 and 1400 h whereas the later in another greenhouse had a peak activity between 1000 and 1200 h.

Bomfim *et al.* (2014) reported that the foraging activity of *Scaptotrigona* sp. fit well into the temporal window of anthesis of greenhouse grown mini watermelon with a period of foraging activity from 0530 to 1400 h and a maximum peak occurring at 0800 h.

The foraging activity of *Melipona marginata* Lepeletier took place throughout the day was most active between 1100 and 1300 h (Kleinert-Giovannini and Imperatriz-Fonseca, 1986).

dos Santos *et al*. (2008) reported the activity period of stingless bees *S.* aff. *depilis* and *N. testaceicornis* as pollinators of cucumber plants (*Cucumis sativus* var. *caipira*) in greenhouses was from 0900 to 1600 h. The former had its maximum collecting activity at 1200 h, whereas later had two activity peaks, the first before noon at 1000 h and the second in the afternoon at 1300 h.

2.1.3 Foraging Behaviour of Stingless Bee in Polyhouse

2.1.3.1 Foraging Rate: Number of Flowers Visited per Unit Time

According to Kakutani *et al.* (1993) the average number of strawberry flowers visited by one stingless bee (*T. minangkabau*) inside the greenhouse for ten minutes was 3.06. Foraging rate of stingless bees (*Nannotrigona perilampoides* Cresson) and bumble bees (*Bombus impatiens* Cresson) under greenhouse condition were compared by Palma *et al*. (2008). The result showed that the average number of tomato flowers visited by stingless bee (5.49) during five minutes was significantly lower than that of bumble bee (10.64). According to Roselino *et al.* (2009), *S.* aff. *depilis* made an average of 45.50 visits during fifteen minutes whereas *N. testaceicornis* made 76.40 visits on strawberries grown under greenhouse condition.

2.1.3.2 Foraging Intensity: Number of Bee Visits per Bloom per Unit Time

In greenhouse grown strawberries, the number of visits by *T. minangkabau* per flower in ten minutes was significantly lower (0.31) than that of honey bee (*A. mellifera*) (0.77) (Kakutani *et al.,* 1993). Palma *et al.* (2008) reported that in enclosed condition the number of visits by *N. perilampoides* (2.31) per tomato flower in five minutes was significantly lower than that of bumble bee *B. impatiens* (8.25).

2.1.3.3 Foraging Speed: Time Spent by Bees per Flower

Palma *et al*. (2008) recorded that the average time spent by *N. perilampoides* on a single tomato (*Lycopersicon esculentum* Mill.) flower was 81.30 sec. which is significantly higher than that of bumble bee *B. impatiens* (24.80 sec.) under protected cultivation. In greenhouse condition, foragers of *S.* aff. *depilis* spent an average 29.20 sec. and *N. testaceicornis* spent an average 73.80 sec. for each flower visit. (Roselino *et al.*, 2009). Nicodemo *et al*. (2013) analysed the duration of visitation of *N. testaceicornis* on greenhouse grown parthenocarpic cucumbers and found that, visits in the morning lasted longer than those that occurred during the afternoon. According to Nunes-Silva *et al.* (2013) the average time spent by *Melipona fasciculata* Smith on eggplant (*Solanum melongena* L.) flower was 96.00 sec. in greenhouse.

Bomfin *et al.* (2014) recorded that the foraging speed for nectar collection by a *Scaptotrigona* sp. forager varied between 2.27 and 43.95 sec. with an average of 13.10 sec. Tej *et al.* (2017) observed the foraging speed of *T. iridipennis* for pollination of cucumber in two different greenhouses revealed that floral handling time varied between the foragers which collected nectar (7.80 sec. in TNAU orchard and 7.40 sec. in Srivilliputhur) and pollen (3.90 sec. in TNAU orchard and 3.50 sec. in Srivilliputhur). Also, the duration of visit was long in case of female flowers compared to male flowers.

2.1.4 Diurnal Variation in Colony Activity

The effect of *N. perilampoides* as a pollinator of tomato was studied and the number of bees returning to the hive with pollen loads increased across the day with clear peak at 1100 h and then decreases. (Cauich *et al.*, 2004; Palma *et al.*, 2008). According to Nunes-Silva *et al.* (2013), stingless bee (*M. fasciculata*) foraging activity (bees exiting the hive and foragers returning with pollen) was most intense in the morning and decreased gradually in the course of the day.

Jaapar *et al.* (2018) observed the number of incoming foragers of stingless bee (*Heterotrigona itama* Cockerell) were low in the morning 0800 h (103.30) and then increase at 1000 h (116.80), were the most pollen foragers observed between 0900 to 1100 h and the nectar foragers observed along the day.

According to Oliveira-Abreu *et al*. (2014), the number of nectar foragers of *M. quadrifasciata* increases along the day and reach a peak during 0830 to 0850 h in the morning and pollen foragers were peaked between 0930 to 0950 h. After this period the income of pollen decreased gradually, while the bees remain collecting nectar for the rest of the day.

2.1.5 Quantity of Pollen Grains Collected by Bee

Pangestika *et al.* (2017) counted the number of pollen grains collected by three different stingless bee species using heamocytometer. Pollen load collected by *H. itama* was 31,392 pollen grains, majority of which belongs to Poaceae flowers (76.49 %). *Lepidotrigona terminata* Smith collected 23,017 pollen grains, majority belonging to Euphorbiaceae flowers (80.46 %). *Tetragonula laeviceps* Smith collected 8,015 pollen grains, 83.33 % of which is from Solanaceae flowers. Similar study conducted by Cholis *et al.* (2020) reported that *T. laeviceps* collected 1,22,594 pollen grains and *H. itama* collected 56,575 pollen grains.

Stanghellini *et al.* (2002) reported that during the time of anthesis staminate flower of cucumber contains an average of 10,539 pollen grains, more than 57.00 per cent of which is removed by the bees two hours following anthesis.

2.1.6 Influence of Weather Parameters on Foraging Behaviour

Heinrich and Esch (1994) reported that the regulation of bee body temperature (thermoregulation), which is mandatory for the flight activity, varies with surrounding temperature. The pattern of foraging activity related to collection of pollen and nectar are also influenced by the climatic factors (Silva and Gimenes, 2014). The number of incoming and outgoing foragers of *Geniotrigona thoracica* (Smith) in Malaysia shows a positive correlation to temperature, relative humidity and light intensity (Sajap *et al*., 2015). According to Soares *et al*. (2019), the flight activity of stingless bees *Trigona spinipes* Fabricius was correlated positively with temperature and negatively with relative humidity.

Heard and Hendrikz (1993) observed that the flight activity of *T. carbonaria* were more influenced by temperature and radiation. The daily activity period was longer in the warmer months (October-March inclusive) of the year (10 to 14 h); radiation was the only variable limiting flight activity both in the morning and evening. The daily flight activity period was comparatively shorter in cooler months (April-September) of the year (3 to 4 h); temperature was the main variable limiting flight activity in the morning but radiation was the limiting variable in the evening. Intensity of daily flight activity was greatest in September and least in May.

Kakutani *et al*. (1993) remarked that there is a parallel relation in between temperature and foraging activity of stingless bee (*T. minangkabau*) on strawberry grown in green house. Contrera *et al.* (2004) claimed that the flight activity of stingless bee *Trigona hyalinata* (Lepeletier) was negatively correlated with temperature and time of the day; while positively correlated with relative humidity and barometric pressure. Palma *et al*. (2008) confirmed that foraging activity of *N. perilampoides* on tomato flowers were affected by temperature, humidity and light intensity. Humidity inside the green house was negatively correlated with the foraging activity while temperature and light intensity were positively correlated.

2.2 FLORAL BIOLOGY OF CUCUMBER

Cucumber (*C. sativus*) flowers are unisexual, pentamerous and axillate. Staminate flowers borne in clusters and pistillate ones are usually borne solitary on a stout peduncle. The pistillate flower is easily recognized by the large ovary at the base of the flower. The staminate flowers produce three stamens. The pistillate flower has three stigma lobes and a short broad style (Heimlich, 1927). The staminate flowers appear about 10 days before pistillate flowers appear. The ratio of male to female flowers is ten to one in monoecious cultivars (Kohli and Vikram, 2005).

According to Primack (1987), the type of flower and amount of rewards offered by the flowers select the type of pollinators. Nunes-Silva *et al.* (2013) suggested while selecting a pollinator for a particular crop, the foraging activity should overlap with the floral receptivity for a successful crop production.

Satheesha (2010) reported the time of anthesis of cucumber during early hour of the day with maximum number of flowers (93.30%) opened during 0600 to 0700 h and the flowers remain opened for a single day. The quantity of nectar in cucumber gradually increased with the advancement of time and reached maximum at 1200 h in both staminate and pistillate flowers. The number of nectaries present was three in both male and female flowers. The receptivity of stigma was maximum at 0800 h and receptivity lost by 50.00 per cent at 1400 h. The length of the corolla tube measured 3.40 cm in male and 3.50 cm in female flowers.

2.3 MORPHOLOGICAL ADAPTATIONS OF BEE IN RELATION TO FLORAL BIOLOGY

Bess and flowers have coevolved millions of years ago and their relationship is a mutual success. They evolved behavioural and physiological adaptations to gather and transport pollen more efficiently. They possess branched body hairs that are densely packed. These specialized hair groups (brushes, combs, and scrapers) help in extraction of hidden pollen and are important in the acquisition of pollen from flowers. They gather the loose pollen from their entire body parts (grooming) and concrete into pollen loads in the specialized transport structures (corbicula) (Thorp, 2000; Shimizu *et al*., 2014).

According to Gilbert (1981) the foraging speed (time spent per flower) and foraging rate (number of flowers visited per minute) depend upon the foraging behaviour of insects and floral structure of the crop concerned, particularly the corolla depth.

There exist complex relationships between tongue length of bees and flower choice. While seeking nectar from flowers, bees preferentially visit plant species with corolla depths which approximately match its own tongue length. Short tongued bees can take nectar from open shallow corolla, or from longer corolla at certain times of day when they filled with nectar. But small bees can crawl right into the corolla tube because of their small body size (Willmer, 2011).

2.4 EFFECT OF STINGLESS BEE POLLINATION ON CROP YIELD

Stingless bees are found to be performed best as a pollinator in enclosures and therefore be a valuable alternative to honey bees for commercial crop pollination (Slaa *et al.*, 2000). Different species of stingless bees are found to effectively pollinate many crops and enhances qualitative as well as quantitative parameters. Stingless bee improves the quality of crops in terms of fruit length, circumference and volume. Per cent of fruit set is found to be enhanced and it also reduces the fruit malformation. (Slaa *et al.*, 2006)

In Japan, Amano (2004) compared the pollination efficiency of stingless bees (*T. carbonaria* and *Scaptotorigona bipunctata* Lepeletier) and honey bees (*A. mellifera*) on white clover, tomato, cucumber, eggplant, paprika and red pepper in greenhouse and apiary areas showed that stingless bees pollinate just as honey bees, apart from the differences in floral biology among the crops. Even in self-pollinated crops, pollination using stingless bees found to increase the number and quality of fruits and also seed production due to the increased transference of pollen grains (Cauich *et al*., 2006).

2.4.1 Qualitative and Quantitative Yield Parameters

2.4.1.1 Fruit Set

The success of pollination results in the fertilization of flowers visited by bees. Cauich *et al.* (2004) found that fruit set was higher for stingless bee (*N. perilampoides*) pollinated tomato plants (83.00 %) when compared to non- pollinated (52.60 %) and mechanical vibrated ones (78.50 %). According to del Sarto *et al*. (2005), the tomato flowers not pollinated did not set any fruit. Palma *et al.* (2008) found higher fruit set for stingless bee (*N. perilampoides*) pollinated tomato plants (80.10 %) compared to mechanically vibrated plants (76.40 %). The largest number of fruits (1414.00 tomatoes) was harvested from the greenhouse with *M. quadrifasciata* compared to the open pollinated ones (730.00 tomatoes) (dos Santos *et al*., 2009).

dos Santos *et al.* (2008) recorded that in both greenhouses containing stingless bee colonies of *S.* aff. *depilis* and *N. testaceicornis*, the yield exceeds 400.00 cucumbers, compared to the greenhouses without pollinators (276.00 and 28.00) and open pollinated plot (321.00). Nicodemo *et al.* (2013) suggested that even in parthenocarpic cucumber variety when bees were excluded, fruit set was 78.00 per cent; however, when bees had access to the flowers, fruit set was significantly (19.20 %) higher. Tej *et al.* (2017) evaluated the pollination efficiency of stingless bee *T. iridipennis* on cucumber cultivated in greenhouses at two different locations *viz*., TNAU and Srivilliputhur. The per cent increase in number of fruits per plant was 2.50 and 3.20 per cent in pollinated plants compared to their respective control (no pollination).

Comparative study, on pollination efficiency of two stingless bees *S.* aff. *depilis* and *N. testaceicornis* on greenhouse strawberries was studied by Roselino *et al.* (2009). The stingless bee pollinated strawberries had higher yield from *S.* aff. *depilis* (743.00) than that of the *N. testaceicornis* (847.00) and control greenhouse (438.00).

Cauich *et al.* (2006) reported that the numbers of *Capsicum chinense* Jacq. fruits produced were 29.00 per cent from the bagged flowers, whereas 89.00 per cent from the stingless bee (*N. perilampoides*) pollinated ones. Nunes-Silva *et al.* (2013) observed that the pollination by *M. fasciculata* increased fruit set of eggplants by 29.50, 32.50, 45.70, and 12.10 per cent compared to the control group without pollination, autogamy, geitonogamy, and cross pollination.

2.4.1.2 Malformed Fruits

According to Kakutani *et al.* (1993) pollination of greenhouse strawberry by the stingless bee, *T. minangkabau*, showed a lower rate of deformed berries (73.00 %) compared to control area (90.00 %). Malagodi-Braga and Kleinert (2004) reported that the percentage of marketable strawberry fruit was higher in stingless bee (*Tetragonisca angustula* (Latreille)) pollinated greenhouse (97.90) compared to open pollination (88.20). Roselino *et al*. (2009) claimed that the percentage of deformed strawberries were very much less (2.00) in both greenhouses with bees (*S.* aff. *depilis* and *N. testaceicornis*) compared to open field (13.00) and greenhouse condition without bees (23.00).

Cruz *et al.* (2005) found that the percentage of malformed fruits was lower for stingless bee (*Melipona subnitida* Ducke) pollinated sweet pepper (*Capsicum annuum* L.) (17.00) compared to self pollinated (48.00) and hand pollinated ones (45.00).

2.4.1.3 Fruit Length

According to Azmi *et al.* (2016) there is a significant increase in the average length (13.00 cm) of chilli fruit produced from *H. itama* pollination, compared to self-pollination (9.02 cm) under protected cultivation. Azmi *et al.* (2017) reported that the cucumber from stingless bee (*H. itama*) pollinated ones were significantly longer (22.20 cm) than the length of cucumber produced by plants without stingless bee pollination (19.61 cm). Tej *et al*. (2017) revealed that stingless bee (*T. iridipennis*) pollinated plots in TNAU orchard had an average fruit length of 19.10 cm compared to 17.60 cm in the control (8.50 % increase) while the pollinated plots in Srivilliputhur had an average fruit length of 18.80 cm compared to 17.30 cm in the control (5.70 % increase).

2.4.1.4 Fruit Diameter

Cruz *et al.* (2005) remarked that the average fruit diameter of sweet pepper (*C. annum*) on stingless bee (*M. subnitida*) pollinated treatments were significantly greater (5.40 cm) than that of the treatment with restricted pollination (4.88 cm). According to Azmi *et al.* (2016) *H. itama* pollination had significantly increased the average fruit diameter of chilli (12.40 mm) than self-pollinated one (11.38 mm).

Azmi *et al.* (2017) reported that in greenhouse, cucumber produced from stingless bee (*H. itama)* pollination were larger in diameter (17.84cm) compared with that of the cucumber from plants without stingless bee pollination (15.75 cm). Tej *et al.* (2017) evaluated the pollination efficiency of stingless bee *T. iridipennis* on cucumber cultivated in greenhouses at two different locations *viz*., TNAU Orchard and Srivilliputhur. The increase in fruit girth was 6.50 and 5.60 per cent in stingless bee pollinated plants compared to their respective control (no pollination).

2.4.1.5 Single Fruit Weight

According to Cauich *et al.* (2004), average fruit weight was higher for stingless bee (*N. perilampoides*) pollinated tomato plants (67.30 g) compared to non-pollinated (61.17 g) and mechanically vibrated ones (69.70 g). Palma *et al.* (2008) claimed that the average fruit weight was higher for stingless bee (*N. perilampoides*) pollinated tomato plants (197.75 g) compared to mechanically vibrate ones (158.00 g).

Malagodi-Braga and Kleinert (2004) found that the mean values of the fresh weight of strawberry grown in greenhouse were higher in *T. angustula* pollination (36.40 g) than in open pollination (25.90 g). Roselino *et al.* (2009) remarked that the strawberries from the greenhouses with *S.* aff. *depilis* and *N. testaceicornis* were heavier (11.20 g and 12.80 g respectively) than those from the greenhouses with no bees (10.60 g)

The study conducted by Cruz *et al*. (2005) reported that the greenhouse grown sweet pepper (*C. annuum*) pollinated by stingless bee, *M. subnitida* gives higher average fruit weight (53.16 g) compared to self-pollination (40.99 g). Nunes-Silva *et al*. (2013) recorded that pollination by *M. fasciculata* significantly increased fruit weight by 96.00 per cent compared to the control group and by 34.00 per cent compared to the autogamy treatment. Studies conducted by Azmi *et al*. (2016) revealed that chilli produced from *H. itama* pollination have significantly heavier fruit (11.60 g) than self-pollination (8.63 g)

With regard to the quality parameters such as fruit size and weight, cucumber collected from two greenhouses with stingless bees *S.* aff. *depilis* and *N. testaceicornis* were significantly larger and heavier than those collected in respective controls (no bees). Moreover, the fruits from the greenhouses without bee pollination did not produce seeds (dos Santos *et al*. 2008).

Azmi *et al.* (2017) found that the cucumber from stingless bee pollination were significantly heavier (0.43 kg) than the weight of cucumber produced from pollination treatment without stingless bee (0.30 kg). Tej *et al*. (2017) reported that stingless bee (*T.*

iridipennis) pollinated plots in TNAU orchard had an average fruit weight of 119.00 g compared to 113.70 g in the control (4.70 % increase) while the pollinated plots in Srivilliputhur had an average fruit weight of 109.90 g compared to 101.20 g in the control (8.60 % increase).

2.4.1.6 Number of Seeds per Fruit

Cauich *et al.* (2004) reported that seed number was higher for stingless bee pollinated tomato plants (200.40) compared to no pollination (120.50). The study conducted by Palma *et al*. (2008) showed the average number of seeds per fruit was higher for stingless bee (*N. perilampoides*) pollinated tomato plants (74.48) compared to mechanical vibration (42.03). dos Santos *et al*. (2009) revealed that tomato pollinated by *M. quadrifasciata* were larger and had more seeds than those produced in the greenhouse with honey bees, in greenhouse without pollinators and open field.

Cruz *et al.* (2005) found that average number of seeds per fruit was higher for stingless bee *M. subnitida* pollinated sweet pepper (*C. annuum*) (137.83) compared to self- pollination (74.16). Cauich *et al.* (2006) remarked that *C. chinense* which is self pollinated require cross pollination to improve yield as well as for high seed production. The average number of seeds per fruit produced in the stingless bee (*N. perilampoides*) pollinated crops (59.80) were higher than self pollinated crops (32.10). Azmi *et al.* (2016) claimed that chilli produced from *H. itama* pollination have higher average number of seeds per fruit (112.54) than self pollination (48.54).

2.4.1.7 Seed Weight per Fruit and Germination Percentage

Brewer (1974) reported that bee pollination is essential for qualitative and quantitative increase in water melon yield including mean seed weight. Sihag (1985) remarked that bee pollination increases seed yield in cruciferous and umbelliferous crops. According to dos Santos *et al*. (2008) the seeds of cucumber produced in the open field (0.15 g) weighed less than those of fruits deriving from bee pollinated flowers in the two greenhouses (0.20 g, 0.25 g). Fruits from the greenhouses without bee pollination did not produced seeds. Shwetha *et al*. (2012) remarked that the number of sound seeds and seed weight of cucumber were higher for *A. cerana* pollination (425.10 and 4.11 g, respectively) compared to *T. iridipennis* pollination (324.94 and 2.84 g, respectively).

According to Ozyigit *et al*. (2015) seed germination percentage of *Stevia rebaudiana* Bertoni was high for open pollination compared to self pollination. Aneetta (2017) reported a higher per cent of seed germination in bee pollination (80.25) compared to that of control (68.75).

2.4.2 Yield

Cauich *et al.* (2004) reported that the fruit production per unit area was higher for stingless bee pollinated tomato plants (5.72 kg) compared to no pollination (3.34 kg) and mechanical vibration (5.66 kg). According to Cauich *et al.* (2006) cross pollination using stingless bee can increase the yield of *C. chinense* by 3.50 kg m^{-2} whereas in self-pollination it was 1.80 kg m^{-2}.

A study by dos Santos *et al*. (2008) showed that the highest cucumber yield (with the highest amount of perfect fruits) was found in those greenhouses which housed the stingless bees *S*. aff. *depilis* and *N.testaceicornis* as pollinators. Nicodemo *et al.* (2013) suggested that placing colonies of stingless bees (*N. testaceicornis*) in the greenhouse promotes 26.00 per cent increase in production of parthenocarpic cucumber cultivar.

2.5 INCIDENCE OF PEST AND DISEASE IN CUCUMBER

Favourable climatic conditions inside the protected cultivation along with continuous monocropping attract pests and diseases. Once these are established, it will be difficult to control the pests and diseases (Gerson and Weintraub, 2007). Amar and Banyal (2011) reported the major disease occurrence of cucumber under protected cultivation in Himachal Pradesh is downy mildew followed by powdery mildew with disease severity of 25.00 to 90.00 per cent and 20.00 to 40.00 per cent, respectively. Disease incidence becomes more severe during the rainy season.

Major pests of cucumber under protected cultivation are sucking pests *viz*., green peach aphid, *Myzus persicae* (Sulzer), mealy bug, *Ferrisia virgata* (Cockerell), thrips, *Astrothrips tumiceps* (Karny), *Thrips hawaiiensis* (Morgan), *Frankliniella schultzei* (Trybom) spotted red mite, *Tetranychus* sp., and leaf feeders *viz*., pumpkin caterpillar, *Diaphania indica* (Saunders), American serpentine leaf miner, *Liriomyza trifolii* (Burgess) (Thamilarasi, 2016).

Materials and Methods

3. MATERIALS AND METHODS

Study on "Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) in cucumber (*Cucumis sativus* L.) under protected cultivation" was carried out at College of Agriculture, Vellayani. The experiments were conducted from November, 2019 to February, 2020. The materials used and methodology adopted for the study is described as follows.

3.1 STUDIES ON FORAGING BEHAVIOUR OF STINGLESS BEES IN POLYHOUSE

Seedlings of non-parthenocarpic salad cucumber (*C. sativus*) variety, AAUC-2 were transplanted and raised through organic farming system according to the adhoc package of practices recommendations for organic farming by Kerala Agricultural University (Alexander *et al.*, 2009). The experiment was conducted with two treatments in a polyhouse (150 m^2) separated by insect proof net (Plate 1).

First treatment was stingless bee assisted pollination where a single bee colony was placed at the center of the treatment plot after initiation of flowering (10 % flowering) (Plate 2). The second treatment was hand pollination (Plate 3) where pollen from male flowers was collected using a fine brush and dusted on the stigma of female flowers.

3.1.1 Mode of Alighting of Individual Foragers Visiting the Bloom

Mode of alighting of fifty stingless bees on male flowers during the peak period of bee activity was observed. Individual foragers alighting directly on top of stamen were considered as top workers while those alighting on petals as side workers (Plate 4).

3.1.2 Initiation and Cessation Time of Foraging Activity

The period of stingless bee foraging activity (visiting the flowers for pollen and nectar) inside the polyhouse was determined from the time of appearance of first forager outside the hive to the time of returning by the last forager. Observations were taken continuously for 10 days.

3.1.3 Foraging Behaviour

The foraging behaviour of stingless bees was recorded during the peak flowering stage of the crop. The observation was taken at hourly intervals during 0800-0900, 0900-1000, 1000-1100, 1100-1200, 1200-1300, 1300-1400, 1400-1500, 1500-1600 and 1600-1700 h of the day and repeated for a week period (Thakur, 2007).

3.1.3.1 Foraging Rate

Foraging rate of stingless bees was recorded as the number of male flowers visited by individual bee in 5 min. The observations were recorded nine times a day as mentioned under 3.1.3.

3.1.3.2 Foraging Intensity

Foraging intensity is the number of individual foragers visited per flower per ten minutes. Foraging intensity in male flowers was recorded during nine time intervals for a week as mentioned under 3.1.3.

3.1.3.3 Foraging Speed

Foraging speed was observed at hourly intervals (3.1.3) in terms of time spent by individual bees on a flower. This is the duration between first alighting on flower and the time at which bee leaves the flower and was recorded with the help of a stop watch. Time spent by pollen foragers on male and nectar foragers on male and female flowers was recorded separately.

(A) Saw toothed polyhouse

(B) Poly house division using insect proof net

Plate 1. Field view of experimental plot

Plate 2. ***Tetragonula iridipennis*** **colony installed in treatment plot**

Plate 3. Hand pollination

(A) Side worker alighting on male flower

(B) Top worker alighting on male flower

(C) Side worker alighting on female flower

(D) Top worker alighting on female flower

Plate 4. Mode of alighting of *Tetragonula iridipennis*

3.1.4 Diurnal Variation in Colony Activity

The total number of returning (pollen and nectar) and outgoing foragers were counted separately for 5 minutes at the entrance of the bee colony. The number of bees was counted from 0800 - 1700 h at hourly intervals.

3.1.4.1 Total Number of Incoming and Outgoing Foragers

The total number of bees entering and exiting the hive was recorded for five minutes separately at varying time periods as mentioned under paragraph 3.1.4.

3.1.4.2 Number of Returning Pollen and Nectar Foragers

The number of bees entering the hive with either pollen or nectar was recorded separately for five minutes at varying time periods as mentioned under paragraph 3.1.4 and repeated for three weeks. Pollen collectors were identified by the pellets of pollen adhering to their hind legs. Those without pollen pellets were considered as nectar gatherers; often their abdomen bulged with nectar.

3.1.5 Number of Pollen Grains Collected by Bees

Pollen foraging bees were captured during different hours of the day, while they enter the hive. The bees were collected in small vials; rinsed the pollen grains adhering all over their body in 1 mL of distilled water. 'Improved Neubauer heamocytometer' was used to count the number of pollen grains collected by bee per flight observing under stereo-zoom microscope (Plate 5).

3.1.6 Influence of Weather Parameters on Foraging Behaviour

Weather parameters such as temperature (°C) and relative humidity (%) inside the polyhouse were measured hourly using a thermohygrometer (data logger) at each observation period mentioned in 3.1.3.

3.1.7 Statistical Analysis

The experiment on foraging behaviour and colony activity were carried out using Completely Randomised Design. Recorded data were subjected to square root transformation wherever necessary. Statistical analysis was carried out using the software WASP version 2.2. Influence of temperature (°C) and relative humidity (%) on foraging behaviour of stingless bees inside the polyhouse were analyzed by correlating them with colony activity. Data were correlated with the aid of OPSTAT and SPSS software.

3.2 FLORAL BIOLOGY OF CUCUMBER

Floral biology of cucumber flowers each of male and female was studied in detail for better interpretation of the foraging activity of the bees. Ten male and female flowers each were tagged at the time of flower bud initiation and observations were taken.

3.2.1. Days to Flower Bud Initiation

Number of days taken from sowing up to flower bud formation by fifty per cent of plants (each male and female bud) was recorded.

3.2.2 Days to Opening of First Male and Female Flowers

Number of days taken from sowing up to fifty per cent flowering (each male and female flower) was recorded.

3.2.3 Flower Diameter

For measuring flower diameter, ten male and female flowers each were selected randomly. Flower diameter was measured as the distance between end points of diagonally opposite petals using meter scale.

3.2.4 Stamen Length

For measuring stamen length, ten male flowers each of same age and size were randomly selected. The petals were excised and exposed stamens were observed under a stereo-zoom microscope. Stamen length was measured by using image analyzing software (Aneetta, 2017) (Plate 6 A).

3.2.5 Stigma Length

Stigma length is the distance from style end to top surface of stigma. Ten female flowers were randomly selected and stigma length was measured under microscope as mentioned in 3.2.4 (Plate 6 B).

3.2.6 Style Length

Style length is the inter-distance between ovary and stigma. It was measured as mentioned in 3.2.4.

3.2.7 Ovary Length

Ten female flowers were randomly selected and ovary length was measured using a meter scale.

3.2.8 Length of Corolla Tube

Petals of cucumber flowers were fused at the base forming a corolla tube. The length of corolla tube of each male and female flower was measured in order to assess their relation with pollinator biology. Length was measured as mentioned in 3.2.4.

3.2.9 Gap between Corolla Wall and Reproductive Part

Ten flowers each of male and female flowers were randomly selected. In male flowers gap between corolla tube and stamen tip was measured. In female flowers gap between corolla tube and stigma tip was measured. They were measured as per the procedure described under 3.2.4.

3.2.10 Time of Anthesis

The time of the day at which the flowers opened were recorded. Ten randomly selected flower buds were tagged and percentage of flowers open at hourly intervals were recorded during morning hours (0600-0700 h, 0700- 0800 h and 0800- 0900 h).

3.2.11 Statistical analysis

The recorded data on floral biology were analysed using statistical parameters like mean, standard deviation and coefficient of variation.

3.3 MORPHOLOGY OF STINGLESS BEE

Morphological characters of stingless bee which help in acclimatizing to pollination were recorded. Ten worker bees of *T. iridipennis* were observed under stereo-zoom microscope. Required characters were measured by using image analyzing software.

3.3.1 Length of Proboscis

Ten stingless bees were collected, killed and proboscis was excised. Proboscis length was measured as distance from mentum to flabellum by using image analyzing software (Plate 7).

3.3.2 Body Length, Head Length and Head Width

Total length of the body, length of head and width of head of worker stingless bee was measured as mentioned in 3.2.2.

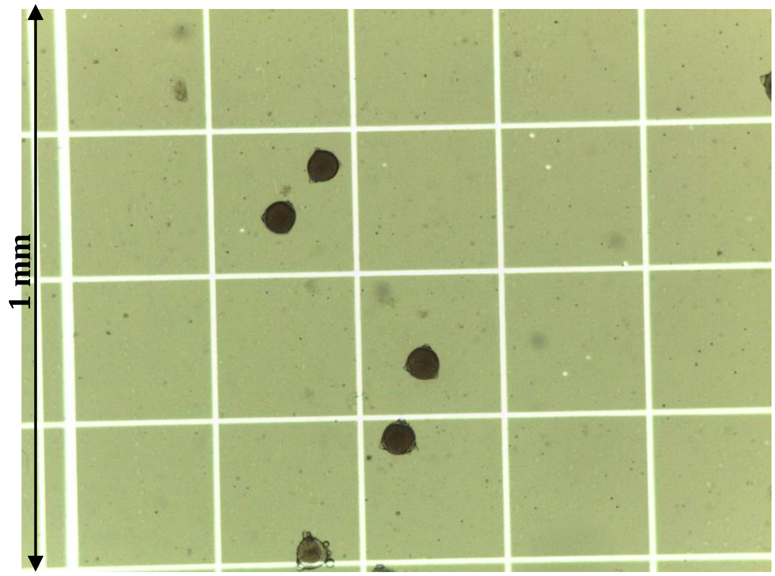


Plate 5. Pollen grains seen under stereo-zoom microscope

(A) Stamen length

(B) Length of stigma and style

Plate 6. Measuring flower characters of cucumber

Plate 7. Proboscis length of *Tetragonula iridipennis*

3.3.3 Statistical analysis

Collected data on bee morphology were analysed by taking arithmetic mean of 10 observations. Standard deviation and coefficient of variation were also calculated.

3.4 EFFECT OF STINGLESS BEE POLLINATION ON CROP YIELD

Effect of stingless bee pollination on crop yield was assessed in terms of the qualitative and quantitative yield parameters (Aneetta, 2017). Observations on these parameters were recorded from both the treatments, stingless bee pollinated and hand pollinated.

3.4.1 Qualitative and Quantitative Yield Parameters

Female flowers were randomly selected in each treatment and tagged after ensuring pollination (hand pollination in one treatment and ensuring bee visit in other treatment).

3.4.1.1 Fruit Set

The per cent fruit set was calculated by using the formula

$$\text{Fruit set \%} = \frac{\text{Total number of fruit set}}{\text{Total number of female flowers tagged}} \times 100$$

3.4.1.2 Malformed Fruit

To calculate the per cent malformed fruits in different modes of pollination, flowers undergone successful pollination was tagged in each treatment. Fruits were harvested at immature stage (8 to 10 days after fruit set) and number of malformed fruits were counted.

$$\text{Malformed fruit \%} = \frac{\text{Total number of malformed fruits}}{\text{Total number of female flowers tagged}} \times 100$$

3.4.1.3 Fruit Length (cm)

The fruits harvested for recording observations on fruit weight were also used for measuring the fruit length and fruit diameter. The polar length of fruit from stalk end to blossom end was measured in cm.

3.4.1.4 Fruit Diameter (cm)

To calculate fruit diameter, fruit was cut opened transversely and fruit width in the middle was measured in cm.

3.4.1.5 Single Fruit Weight (g)

To calculate fruit weight in different modes of pollination, female flowers undergone successful pollination was tagged in each treatment (ten numbers each). These tagged fruits were harvested at 8 to 10 days after fruit set and weight was recorded.

3.4.1.6 Number of Seeds per Fruit

Seeds were extracted from 10 matured ripen fruits (30 days after fruit set) from each treatments. They were soaked overnight in water, washed and dried to obtain seeds. The number of seeds per fruit was counted.

3.4.1.7 Seed Weight per Fruit (g)

Seeds were extracted from 10 matured ripen fruits of each treatments as mentioned in 3.2.6. Weight of seeds per fruit was recorded.

3.4.1.8 Germination Percentage

Seeds obtained from each treatment as per paragraph 3.2.6 were subjected to germination test. Wet paper method was used for studying germination per cent. Hundred seeds were selected from bulked seed lots for each treatment and were placed on moistened filter paper in petri plates. Seeds germinated were counted 2 Days After Sowing (DAS) and germination percentage was calculated as follows.

$$\text{Germination per cent} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

3.4.2 Yield (kg m^{-2})

Total yield per plot in each treatments were recorded separately by combining all the harvests. Yield per plot was converted to yield per unit area using the formula

$$\text{Yield} = \frac{\text{Total yield}}{\text{Total area}}$$

3.4.3 Statistical analysis

The quantitative and qualitative parameters of crop yield from both the treatments (stingless bee pollinated and hand pollinated) were compared using ‘paired t test’.

3.5 INCIDENCE OF PESTS AND DISEASES IN CUCUMBER

The incidence of pests and diseases, if any, were also observed during the period of study.

Result

4. RESULTS

The research on “Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) in cucumber (*Cucumis sativus* L.) under protected cultivation” was carried out in the polyhouse at College of Agriculture, Vellayani, from November, 2019 to February, 2020. The results of the experiment are presented in this chapter.

4.1 FORAGING BEHAVIOUR OF *T. iridipennis.*

4.1.1 Mode of Alighting

On approaching a flower, individual bees paused and made a hovering above the flower before alighting. Two different methods of alighting were observed for *T. iridipennis* foragers while pollinating cucumber flowers in polyhouse. Most of the observed bees (78.00 %) were side workers, on reaching a flower (staminate or pistillate) use the petals as a landing platform, and then walked towards the center of the flower. They walked around the reproductive parts (stamen or stigma) of the flower and seek the nectaries. Finally the bee insert its head inside the corolla tube through the space between corolla tube and reproductive part and reach the nectaries at the bottom.

While the remaining bees (22.00 %) were top workers, on recognizing a flower (staminate or pistillate) made a hovering above the flower and landed directly on the top of stamen or stigma (Figure 1). Then they insert their body into the corolla tube to reach the nectaries at the base.

Irrespective of the way of approach, different body parts of bee including head, thorax, wings, abdomen and legs were dusted with pollen grains. They flew above the flowers, made a hovering and collected the pollen grains which adhere on their body parts and filled the corbicula. Again, they landed the same flower for further collection of nectar and pollen. Same bees were observed to perform both the modes of alighting on same flower during the same visit.

4.1.2 Initiation and Cessation Time of Foraging Activity

Foraging activity of stingless bee occurs throughout the day. The foragers were observed to visit the cucumber flowers from 0750 h in the morning and continued till 1730 h in the evening.

4.1.3 Foraging Behaviour of *T. iridipennis* on Cucumber Grown in Polyhouse

Observations on number of male flowers visited by bee in 5 min (foraging rate), number of bees visited per male flower for 10 min (foraging intensity) and time spent by bee per male flower (foraging speed) were recorded at hourly intervals starting from 0800 - 0900 to 1600 - 1700 h of the day. Significant differences in foraging behaviour were observed at different hours of the day. The results obtained are presented in the Table 1.

4.1.3.1 Foraging Rate

Foraging rate was the highest during 0800 – 0900 h of the day (6.80 male flowers 5 min^{-1}) and found to be on par with 1400 – 1500 h (6.00 male flowers 5 min^{-1}). During 0900 – 1000 h, the foraging rate was recorded as 5.20 male flowers 5 min^{-1} which is on par with 1400 – 1500 h (6.00 male flowers 5 min^{-1}). Foraging rate at 1000 – 1100, 1100 – 1200, 1200 -1300 and 1500 – 1600 h were found to be 4.20, 4.20, 4.00 and 4.00 male flowers 5 min^{-1}, respectively and were on par with each other. The least foraging activity were observed during 1600 – 1700 h with 3.60 male flowers 5 min^{-1} and was on par with 1300 – 1400, 1000 – 1100, 1100 – 1200, 1200 – 1300 and 1500 – 1600 h (4.00, 4.20, 4.20, 4.00 and 4.00 male flowers 5 min^{-1}, respectively).

4.1.3.2 Foraging Intensity

Maximum foraging intensity was observed as 4.40 bees male $flower^{-1}$ 10 min^{-1} during 1000 – 1100 h which is on par with 0900– 1000, 1100 – 1200, 1200 – 1300, 1300 – 1400 and 1400 – 1500 h of the day with 3.00, 2.80, 3.00, 2.80 and 2.80 bees male $flower^{-1}$ 10 min^{-1}. Minimum foraging intensity of 0.80 bees male $flower^{-1}$ 10 min^{-1} was

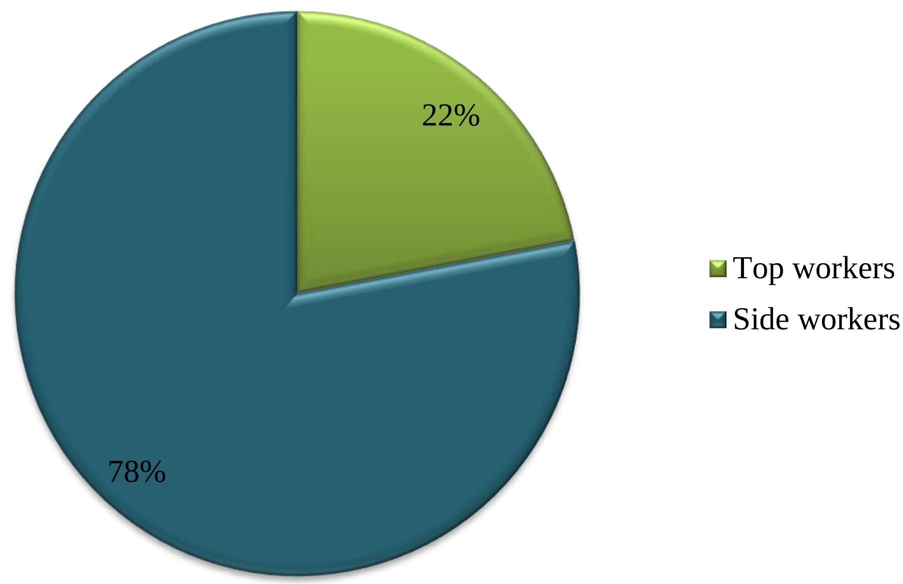


Figure 1. Mode of alighting of stingless bees on flowers

Total number of bees observed = 50

Table 1. Diurnal variation in foraging behavior of *Tetragonula iridipennis* in cucumber grown inside polyhouse

Time period (h)	*Foraging rate (Male flowers 5 min^{-1})	* Foraging intensity (Bees male $flower^{-1}$ 10 min^{-1})	*Foraging speed (sec.)
0800-0900	6.80 $(2.69)^{a}$	1.40 $(1.27)^{c}$	23.20^{bc}
0900-1000	5.20 $(2.38)^{bc}$	3.00 $(1.84)^{a}$	28.00^{abc}
1000-1100	4.20 $(2.15)^{cd}$	4.40 $(2.18)^{a}$	33.20^{ab}
1100-1200	4.20 $(2.16)^{cd}$	2.80 $(1.78)^{ab}$	41.00^{ab}
1200-1300	4.00 $(2.11)^{cd}$	3.00 $(1.81)^{a}$	44.20^{a}
1300-1400	4.00 $(2.10)^{d}$	2.80 $(1.80)^{a}$	39.40^{ab}
1400-1500	6.00 $(2.54)^{ab}$	2.80 $(1.79)^{ab}$	28.20^{abc}
1500-1600	4.00 $(2.11)^{cd}$	1.20 $(1.29)^{bc}$	23.80^{bc}
1600-1700	3.60 $(2.01)^{d}$	0.80 $(1.08)^{c}$	12.00^{c}
CD (0.05)	(0.278)	(0.503)	18.554

*Mean of 5 observations

Figures in parenthesis are square root transformed value recorded during 1600 – 1700 h and is on par with 1500 – 1600 and 0800 – 0900 h of the day (1.20 and 1.40 bees male flower^{-1} 10 min^{-1} respectively).

4.1.3.3 Foraging Speed

Longest time spent by pollen foragers per male flower was recorded as 44.20 sec. during 1200 – 1300 h of the day and was on par with 0900 – 1000, 1000 – 1100, 1100 – 1200, 1300 – 1400 and 1400 – 1500 h (28.00, 33.20, 41.00, 39.40 and 28.20 sec., respectively). At 1500 – 1600 h, the foraging speed was recorded as 23.80 sec. per male flower which is on par during 0800 – 0900 h of the day (23.20 sec. per male flower). Least time spent by pollen foragers per male flower (12.00 sec.) was during 1600 – 1700 h of the day.

Individual bees collecting nectar were found to insert their head inside the corolla tube to reach the nectar at the bottom. They are distinguished from pollen foragers by the lack of pollen storage at the corbicula. The time spent by nectar foragers on male and female flowers during different time intervals of the day are plotted in Figure 2.

The time spent by nectar foraging bee on male flowers varies significantly throughout the day. Maximum time spent by nectar foragers on male flowers was recorded as 141.20 sec. during 0800 – 0900 h which is on par with 112.60 sec. during 1000 – 1100 h. The minimum duration was recorded during 1400 – 1500 h (75.20 sec.) and is on par with 1200 – 1300 h (92.00 sec.).

Irrespective of the time period, nectar foragers spent more time on female flower compared to that of male flowers. The time spent by nectar foragers on female flowers show significant variation at different time intervals of the day. Longest time spent by bee was recorded as 323.40 sec. during 1200 – 1300 h which is on par with 244.80 sec. observed during 1000 – 1100 h. This was followed by 196.80 sec. during 1400 – 1500 h. Shortest time spent by bee was 142.20 sec. on 0800 – 0900 h.

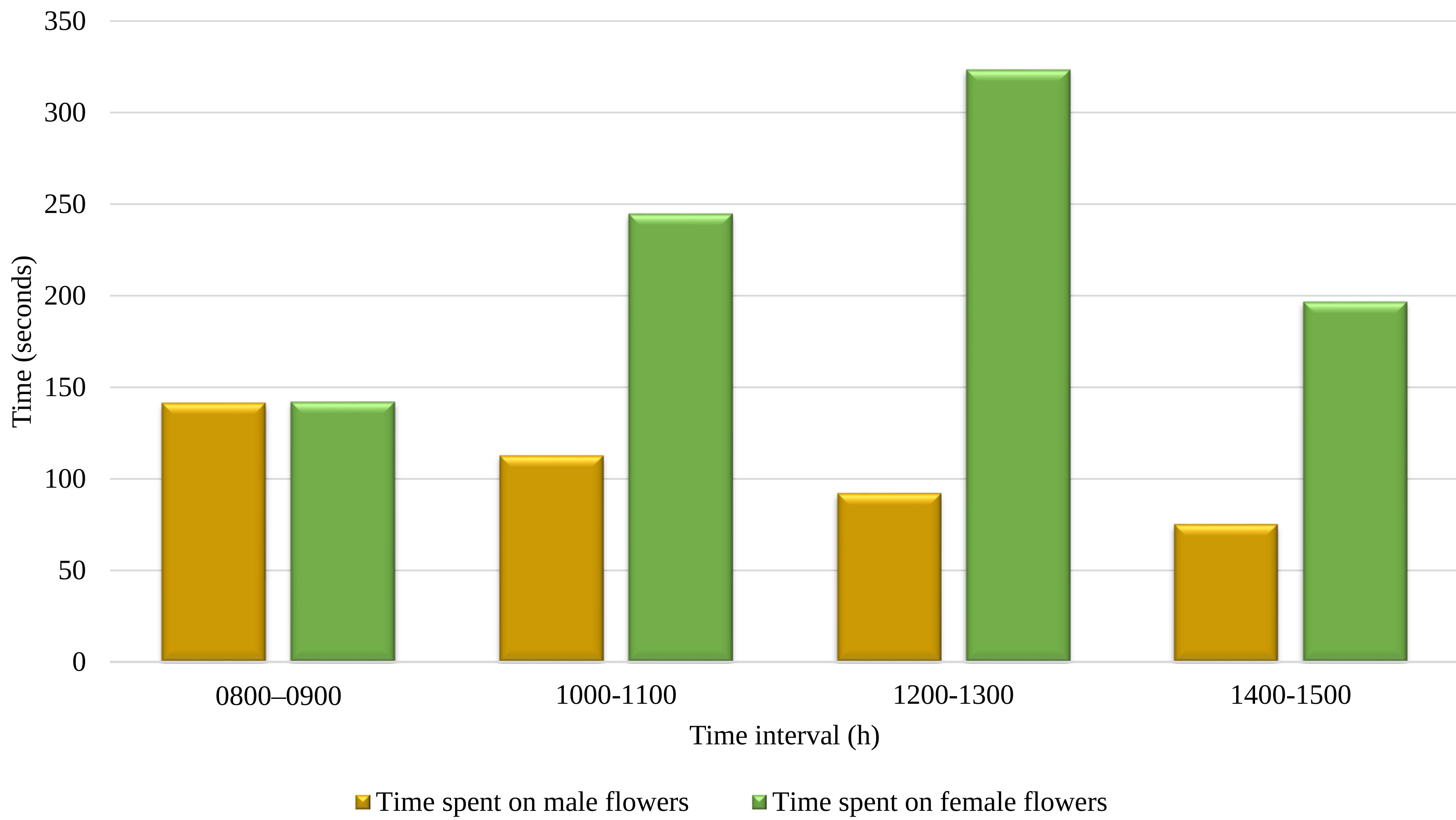


Figure 2. Diurnal variation in time spent by nectar foragers in cucumber flower

4.1.4 Diurnal Variation in Colony Activity

The total number of incoming and outgoing foragers at the hive entrance was counted for 5 minutes during different time intervals of the day. These flight activities differ significantly during different time period. The results obtained are recorded in the Table 2.

4.1.4.1 Total Number of Incoming and Outgoing Foragers

The total number of returning foragers increases along the day with maximum number of bees (67.00) recorded at 1300 – 1400 h and is on par with 45.66 bees recorded during 1200 – 1300 h of the day. Thereafter a declining trend in number of returning foragers was observed. At 1400 – 1500 h an average of 40.66 bees returned, which is on par with 1100 – 1200 h, during which 40.00 bees were counted. A mean of 24.60 bees were counted during 1500 – 1600 h which is followed by 1000 – 1100, 1600 – 1700 and 0900 – 1000 h of the day with 19.33, 9.33 and 8.00 bees, respectively. Least number of incoming bees were recorded during early hour of the day (0800- 0900 h) with a mean of 3.33 bees.

Maximum number of outgoing foragers was counted during 1300 – 1400 h (58.00 bees) which is followed by 1200 – 1300, 1100 – 1200, 1400 – 1500 and 1500 – 1600 h (39.66, 38.33, 32.66 and 30.00 bees, respectively) which were statistically on par. The next highest number of outgoing bees were counted during 1000 – 1100 h (22.66 bees) followed by 0900 – 1000 and 1600 -1700 h (8.66 and 4.66 respectively). Minimum number of outgoing foragers was counted during early hours of the day (0800 – 0900 h) as 2.33 bees.

Table 2. Diurnal variations in colony activity of *Tetragonula iridipennis* in polyhouse

Time period (h)	*No. of incoming foragers	*No. of outgoing foragers
0800-0900	3.33 (1.79)^{f}	2.33 (1.49)^{e}
0900-1000	8.00 (2.90)^{ef}	8.66 (3.02)^{d}
1000-1100	19.33 (4.43)^{de}	22.66 (4.77)^{c}
1100-1200	40.00 (6.34)^{bc}	38.33 (6.21)^{b}
1200-1300	45.66 (6.69)^{ab}	39.66 (6.33)^{b}
1300-1400	67.00 (8.19)^{a}	58.00 (7.64)^{a}
1400-1500	40.66 (6.34)^{bc}	32.66 (5.75)^{b}
1500-1600	24.60 (5.01)^{cd}	30.00 (5.50)^{bc}
1600-1700	9.33 (2.94)^{ef}	4.66 (2.25)^{de}
CD (0.05)	(1.559)	(0.905)

*Mean of 3 observations

Figures in parenthesis are square root transformed value

4.1.4.2 Returning Foragers with Pollen and Nectar

The number of foragers returning to the hive with either pollen or nectar was counted from 0800 – 1700 h at hourly intervals (Plate 8). The observations were taken for 3 weeks; results of the experiment are presented in Table 3.

During the first week, number of pollen foragers was significantly highest during 1000-1100 and 1200 - 1300 h with a similar means of 8.66 bees and were on par during 0900-1000 h (5.33 bees). The second highest number was obtained during 1300 – 1400 h (4.00 bees), followed by the remaining time intervals (0800-0900, 1100-1200, 1400-1500, 1500-1600 and 1600-1700 h with mean of 2.66, 2.66, 3.00, 2.33 and 2.33, respectively) and were on par.

Maximum number of bees collecting nectar were obtained during 1300-1400 h (17.66 bees) followed by 0900-1000, 1000-1100, 1200-1300, 1400-1500, 1500-1600 and 1600-1700 h (9.00, 7.33, 7.00, 5.66, and 4.66 bees, respectively) in the first week, which were on par with each other. Minimum number of nectar foragers recorded was 3.33 during 0800-0900 h, which is statistically on par with 1100-1200 h (4.00 bees).

The number of pollen foragers were the highest (7.33 bees) during 1200-1300 h in second week. The number of pollen collectors decreases before and after this time. An average of 4.00 bees were observed at 1300-1400 h, which were statistically on par with the bees (2.00, 2.66, 1.66 and 2.00) recorded during 0900-1000, 1000-1100, 1100-1200 and 1500-1600 h, respectively. The lowest mean of 1.33 bees were observed during 0800-0900, 1400-1500 and 1600-1700 h.

During second week of observation, the largest number of nectar foragers (19.66 bees) was observed during 1200-1300 h. The mean number of nectar foragers decreases before and after this time. Second largest mean recorded were 12.00 bees during 1300-1400 h which is on par with 1400-1500, 1500-1600, 1100-1200 and 1000-1100 h with mean of 8.66, 6.66, 6.66 and 5.33 bees, respectively. Smallest number of nectar foragers (1.66) was recorded during early hour (0800-0900 h) of the day.

Table 3. Pollen and nectar foraging behaviour of *Tetragonula iridipennis*

Time Period (h)	WEEK 1		WEEK 2		WEEK 3	
	*No. of pollen foragers	*No. of nectar foragers	*No. of pollen foragers	*No. of nectar foragers	*No. of pollen foragers	*No. of nectar foragers
0800-0900	2.66 $(1.76)^{c}$	3.33 $(1.79)^{c}$	1.33 $(1.34)^{c}$	1.66 $(1.24)^{d}$	1.33 $(1.26)^{de}$	3.00 $(1.71)^{f}$
0900-1000	5.33 $(2.34)^{abc}$	9.00 $(2.99)^{b}$	2.00 $(1.58)^{bc}$	5.33 $(2.29)^{c}$	3.66 $(2.03)^{cde}$	7.00 $(2.63)^{ef}$
1000-1100	8.66 $(3.01)^{a}$	7.33 $(2.47)^{bc}$	2.66 $(1.77)^{bc}$	5.33 $(2.30)^{bc}$	8.66 $(3.02)^{ab}$	11.66 $(3.40)^{de}$
1100-1200	2.66 $(1.76)^{c}$	4.00 $(1.95)^{c}$	1.66 $(1.46)^{bc}$	6.66 $(2.57)^{bc}$	6.66 $(2.67)^{abc}$	29.00 $(5.37)^{bc}$
1200-1300	8.66 $(2.96)^{ab}$	7.00 $(2.62)^{bc}$	7.33 $(2.73)^{a}$	19.66 $(4.43)^{a}$	9.66 $(3.04)^{ab}$	36.00 $(5.93)^{b}$
1300-1400	4.00 $(2.08)^{bc}$	17.66 $(4.18)^{a}$	4 $(2.09)^{b}$	12.00 $(3.32)^{b}$	12.00 $(3.52)^{a}$	55.00 $(7.39)^{a}$
1400-1500	3.00 $(1.78)^{c}$	5.66 $(2.35)^{bc}$	1.33 $(1.34)^{c}$	8.66 $(2.85)^{bc}$	4.33 $(2.18)^{bcd}$	36.33 $(5.95)^{b}$
1500-1600	2.33 $(1.64)^{c}$	5.66 $(2.32)^{bc}$	2.00 $(1.55)^{bc}$	6.66 $(2.54)^{bc}$	4.33 $(2.10)^{bcde}$	20.33 $(4.50)^{cd}$
1600-1700	2.33 $(1.64)^{c}$	4.66 $(2.15)^{bc}$	1.33 $(1.29)^{c}$	3.66 $(1.86)^{cd}$	1.00 $(1.17)^{e}$	8.66 $(2.76)^{ef}$
CD (0.05)	(0.899)	(1.022)	(0.635)	(1.027)	(0.950)	(1.299)

*Mean of 3 observations (No. of returning foragers in 5 minutes)

Figures in parenthesis are square root transformed

(A) Incoming forager

(B) Pollen forager

(C) Nectar forager

Plate 8. Foragers of *Tetragonula iridipennis*

Regarding the observations on third week, the highest number of pollen foraging bees (12.00) was observed during 1300-1400 h which is on par with 1000-1100, 1100-1200 and 1200-1300 h (8.66, 6.66, and 9.66 bees, respectively). The lowest number of pollen collectors was observed during 1600-1700 with a mean of one bee.

During third week the peak period of nectar collection was 1300-1400 h with an average of 55.00 bees. The second best period of nectar collection was recorded as 1200-1300 h (36.00 bees) and 1400-1500 h (36.33 bees) which were on par with 1100-1200 h (29.00 bees). The lowest mean were 3.00 bees recorded during 0800-0900 h of the day.

4.1.5 Number of Pollen Grains Adhering to the Body of Bee at Different Time Intervals

Observations on number of pollen grains, that a bee can collect during different hours of the day was recorded (Figure 3). The amount of pollen grains removed by the bee per visit significantly varies along the day.

The number of pollen grains collected by bees varies from 900 to 7300 pollens. During 1000-1100 h, highest average numbers of pollen grains (3566.66) were collected ranging from 2600 to 4400. This is on par with 0800- 0900 h and 1000-1100 h. Mean number of pollen grains collected during 0800- 0900 h was 3050.00 ranging from 2200 to 3600. Mean number of pollen grains collected during 1200 - 1300 h was 3200.00 ranging from 900 to 7300. Lowest number of pollen grains collected (1183) was during 1400-1500 h ranging from 900 to 1500.

4.1.6 Influence of Weather Parameters on Foraging Activity of *T. iridipennis*

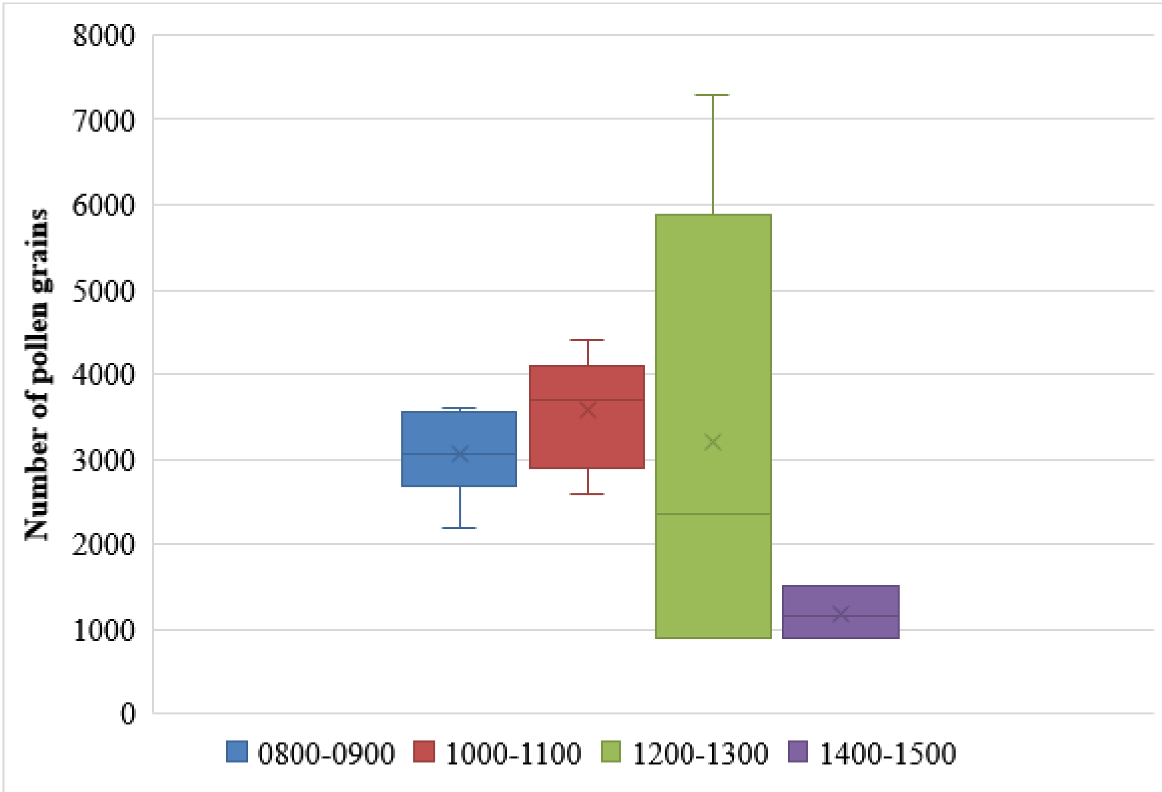
Correlation matrix table (Table 4) shows the correlation between weather parameters *viz.*, temperature and relative humidity on number of returning foragers of *T. iridipennis* at the hive. There was a significant positive correlation between temperature and number of incoming foragers where the correlation coefficient obtained was 0.78. While a negative correlation was observed between relative humidity and number of incoming foragers and the correlation coefficient obtained was -0.68.

The dependence of number of incoming foragers and temperature inside the polyhouse is shown in Figure 4. About 46.00 per cent variation in number of incoming foragers were influenced by changes in temperature (R^2 = 0.46). While 36.20 per cent variation in number of incoming foragers were influenced by changes in relative humidity (Figure 5) inside the polyhouse (R^2 = 0.36).

Table 4. Influence of weather parameters on foraging activity of *Tetragonula irridipennis*

-	Temperature (^{o}C)	Relative Humidity (%)	Number of returning foragers 5 min^{-1}
Temperature (^{o}C)	1	-0.969*	0.78*
Relative Humidity (%)	-0.969*	1	-0.68*
Number of returning foragers 5 min^{-1}	0.78*	-0.68*	1

*Significant difference



Time interval (h)

Figure 3. Number of pollen grains adhering to the body of bees at different time intervals

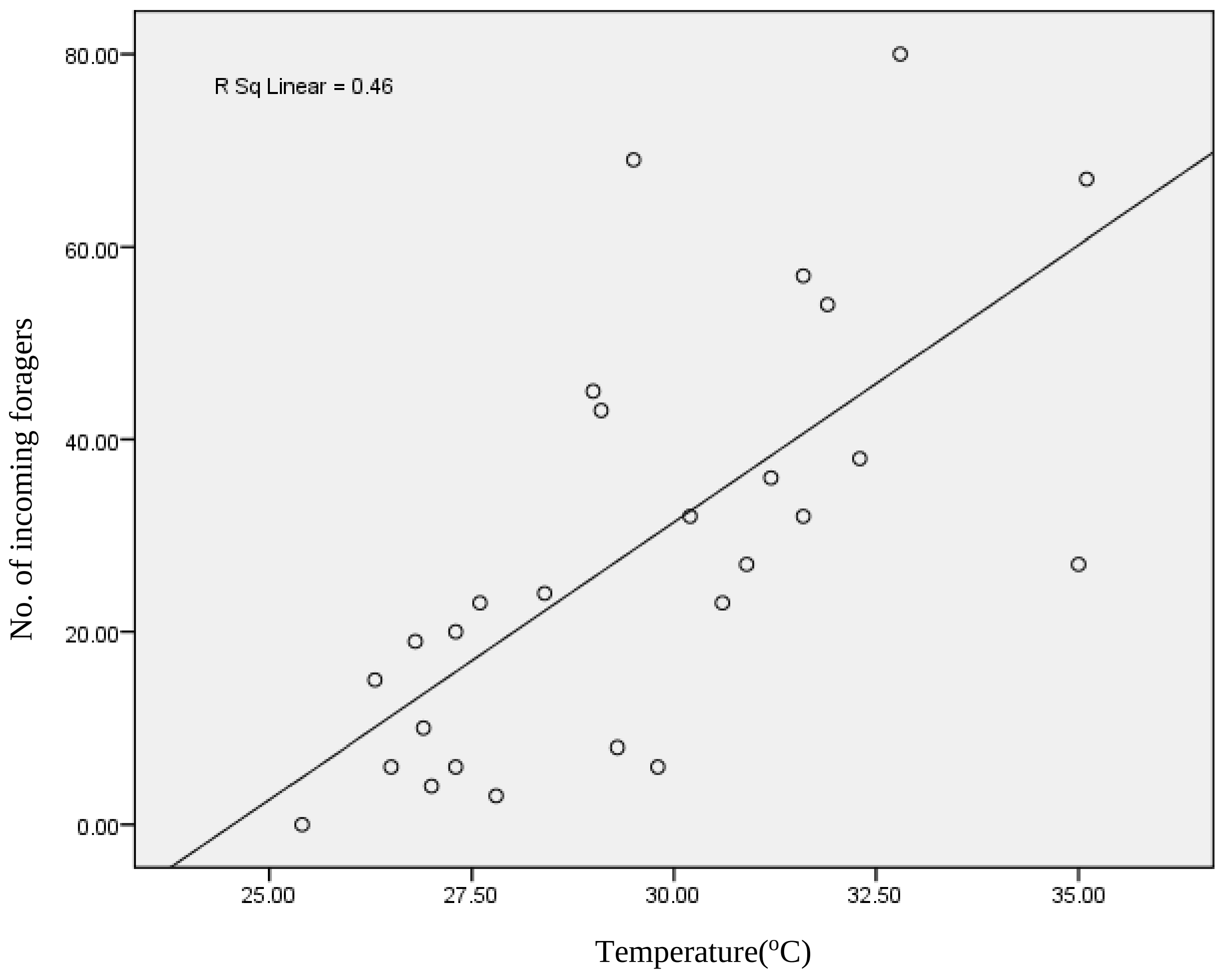


Figure 4. Influence of temperature on foraging activity of *Tetragonula iridipennis*

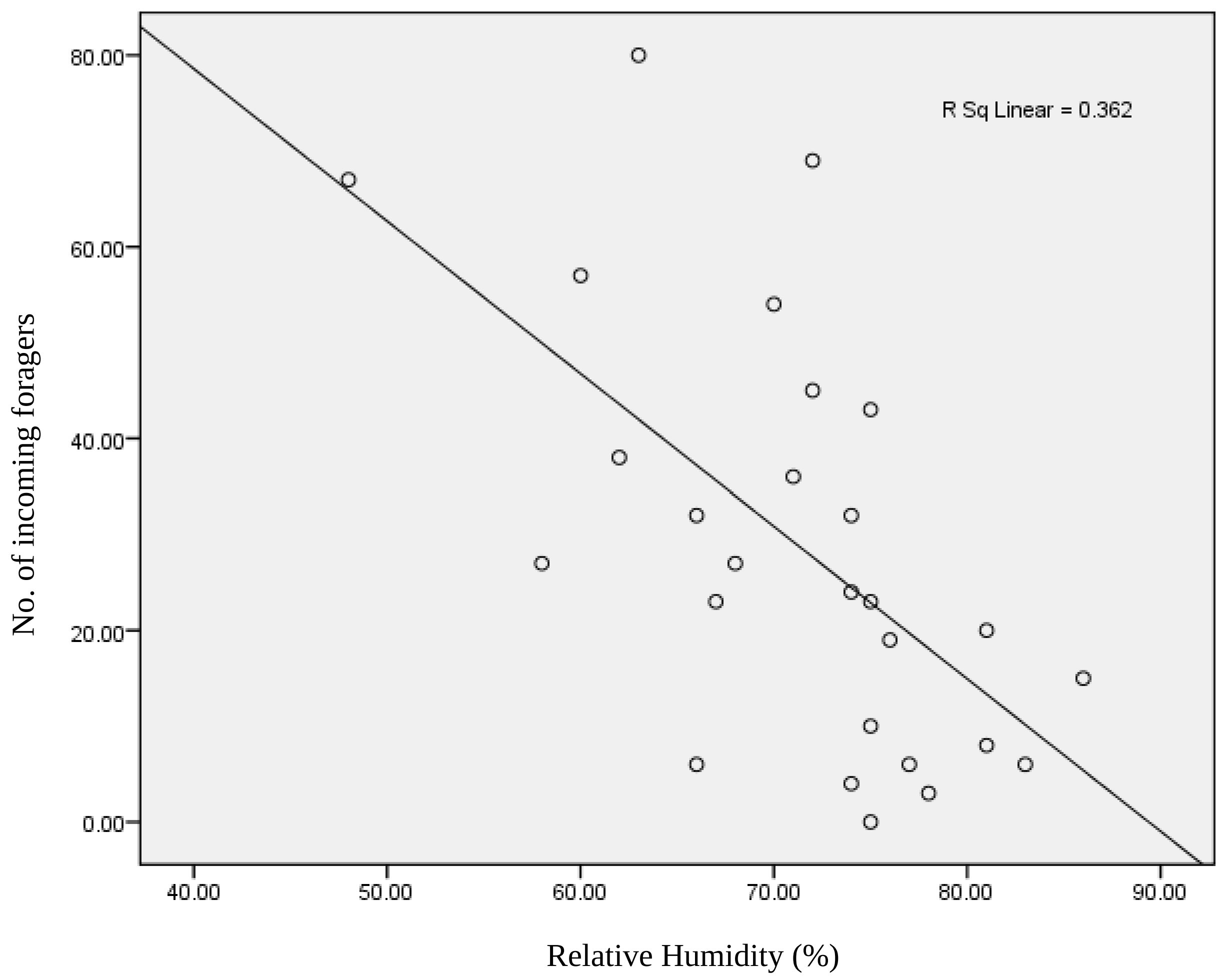


Figure 5. Influence of relative humidity on foraging activity of *Tetragonula iridipennis*

4.2 FLORAL BIOLOGY OF CUCUMBER

Cucumber plant is an annual, tap rooted, succulent, tendril bearing (one per node), climber which grows upon trellis or other supporting frames, with the support of their spiraling tendrils.

Flowers are yellow in colour, bracteate, pedicellate, radially symmetrical, unisexual, pentamerous, epigynous, sepals: five, united, often deeply five lobed; petals: five, united to form a tube, often deeply five lobed and are produced in the axes of leaves.

The male flower bud appeared 25 Days After Sowing (DAS), 8 days prior to that of female flower bud initiation (34 DAS). The staminate and pistillate flowers bloomed, 31 DAS and 39 DAS, respectively (Table 5).

Table 5. Plant and flower characteristics of C*ucumis sativus*

Plant and flower parameters		Mean
Location of flower		Leaf axil
Days to flower bud initiation	Male	25 DAS
	Female	34 DAS
Days taken to flowering	Male	31 DAS
	Female	39 DAS
Flower colour		Yellow
Calyx: No. of sepals		5.00
Corolla : No. of petals		5.00
No. of stamens		5.00
No. of stigma		6.00
Location of nectaries in flower	Male	Base of the stamen
	Female	Base of the style
Forage available	Male	Pollen and nectar
	Female	Nectar
Flower duration (day)		1

DAS- Days after sowing

The size of staminate flowers ranged from 3.80 to 5.60 cm with a mean flower diameter of 4.35 cm which were born either singly or in cluster. Pistillate flowers were larger and ranged from 3.80 to 5.50 cm with a mean diameter of 4.63 cm (Table 6). They are often solitary and had shorter and stouter pedicel than staminate flowers. The pistillate flower is easily recognized by their large ovary at the base of the flower.

Male flowers have five stamens with twisted anthers, varies from 3.86 to 6.00 mm in length with a mean length of 5.02 mm.

Female flowers have six stigmas, with an average length of 2.97 mm in length. The maximum and minimum length of style is 3.54 and 2.80 mm respectively with an average of 3.15 mm. The size of tricarpellary long inferior ovary ranged from 2.40 to 4.30 cm having an average length of 3.30 cm.

The nectaries are present at the base of the stamen in male flowers and at the base of the style in female flowers (Plate 9). The nectaries form a bowl like structure in male flowers, whereas it surrounds the style forming a trough for accumulation of nectar in female flowers. This nectar position helps the pollinators to easily access female nectar. The bees visit the flowers for collecting floral rewards in return to the pollination services. They receive nectar from female flowers; while they receive nectar and pollen from male flowers.

Anthesis of maximum number of flowers (87.00 %) was observed during early hours of the day (0600 – 0700 h). Remaining buds continued to open for another hour and no anthesis was observed after 0800 h (Table 7). The flowers remained open for a single day.

Table 6. Floral biology of *Cucumis sativus*

Parameter	*Flower diameter; Male (cm)	*Flower diameter; Female (cm)	*Stamen length (mm)	*Stigma length (mm)	*Style length (mm)	*Ovary length (cm)
Maximum value	5.60	5.50	6.00	3.55	3.54	4.30
Minimum value	3.80	3.80	3.86	2.10	2.80	2.40
SD	0.52	0.50	0.58	0.44	0.22	0.17
Mean	4.35	4.63	5.02	2.97	3.15	3.31
CV	12.08	10.87	11.64	14.92	7.26	16.57

*Mean of 10 observations

Table 7. Time of anthesis in cucumber in polyhouse

Total number of flowers observed	Percentage of flowers open at hourly intervals		
	0600 – 0700 h	0700 – 0800 h	0800 – 0900 h
10	90	10	0
10	80	20	0
10	90	10	0
Mean	87	13	0

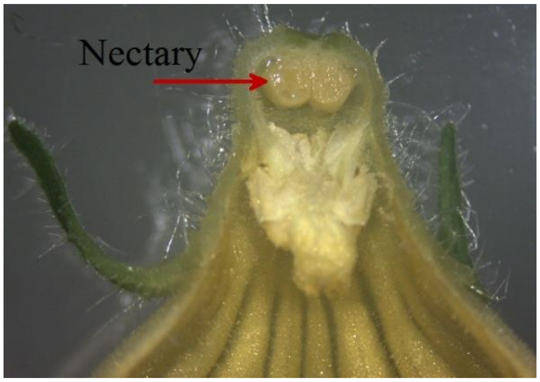
4.3 MORPHOLOGICAL ADAPTATIONS OF BEE IN RELATION TO FLORAL BIOLOGY

Observations on the parameters of floral biology of cucumber *viz*., length of corolla, gap between the wall of corolla and stamen tip in the male flower and gap between the wall of corolla and stigma tip in the female flower and morphology of stingless bee like body length, head length, head width and proboscis length were recorded to assess the adaptation of bees in relation to floral biology. The data are presented in Table 8.

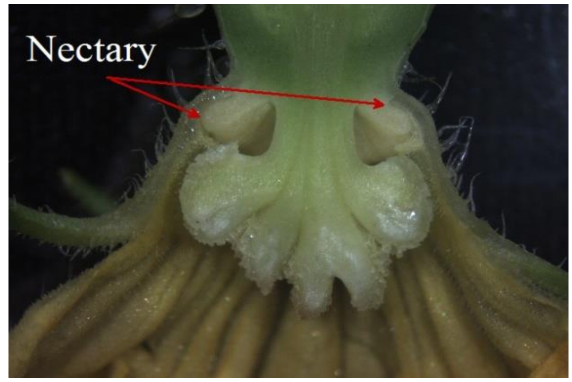
Individual bees (nectar foragers) collect nectar by inserting their head into the corolla tube of both male and female flowers to reach the nectaries (Plate 10).

The length of corolla tube of male flowers varies from 4.50 to 7.70 mm with a mean of 6.14 mm. Maximum and minimum length of corolla tube of female flowers was 6.04 and 4.80 mm, respectively with a mean length of 5.23.

The gap between corolla tube and stamen tip ranges from 0.88 to 2.00 mm having a mean recorded as 1.35 mm. The gap between corolla tube and stigma tip varies from 1.45 to 2.55 mm having an average of 1.91 mm.



(A) Nectary of male flower



(B) Nectary of female flower

Plate 9. Location of nectaries in cucumber flower

(A) Nectar forager in male flower

(B) Nectar forager in female flower

Plate 10. Nectar foragers insert their head inside the corolla tube to reach the nectaries

Table 8. Morphological adaptations of *Tetragonula iridipennis* in relation to floral biology

Parameter	*Corolla tube length (male flower) (mm)	*Corolla tube length (female flower) (mm)	*Gap between corolla tube and stamen tip (mm)	*Gap between corolla tube and stigma tip (mm)	*Proboscis length (mm)	*Body length (mm)	*Head length (mm)	*Head width (mm)
Maximum value	7.70	6.04	2.00	2.55	1.37	4.00	1.50	1.80
Minimum value	4.50	4.80	0.88	1.45	1.19	3.04	1.30	1.70
SD	0.86	0.47	0.43	0.38	0.07	0.28	0.06	0.03
Mean	6.14	5.23	1.35	1.91	1.30	3.69	1.39	1.74
CV	14.00	9.08	31.66	20.22	5.49	7.73	4.49	1.89

*Mean of 10 observations

The body length of bee ranges from 3.04 to 4.00 mm with an average of 3.69 mm. The head length varies from 1.30 to 1.50 mm with an average of 1.39 mm while the width of the head varies from 1.70 to 1.80 mm with an average of 1.74 mm. The length of proboscis varies from 1.19 to 1.37 mm with an average of 1.30 mm.

4.4 EFFECT OF STINGLESS BEE POLLINATION ON CROP YIELD

4.4.1 Qualitative and Quantitative Yield Parameters

Qualitative and quantitative yield parameters of *C. sativus viz*., fruit set, number of malformed fruits, fruit length, fruit diameter, single fruit weight, number of seeds per fruit, seed weight per fruit and germination per cent are detailed in Table 9.

Significant difference was observed in per cent fruit set between stingless bee pollinated and hand pollinated crop. Highest per cent of fruit set was recorded in stingless bee pollinated crop (76.00) compared to that of the hand pollinated one (54.00).

No significant variation was observed between the two treatments in the percentage malformed fruits, fruit length and fruit diameter. The per cent of malformed fruits was 16.10 and 14.00 while the average fruit length was 26.07 cm and 25.99 cm for the stingless bee pollinated crop and hand pollinated crop respectively. The diameter of fruit from polyhouse with stingless bee pollination and hand pollination were on par with a mean of 7.18 cm and 6.81 cm respectively.

The mean weight of single fruit was significantly higher in polyhouse with stingless bee (685.00 g) compared to polyhouse with hand pollination (555.00 g). Number of seeds per fruit was highest for stingless bee pollination (344.10) while for the hand pollinated one, it was 210.90. Average weight of seeds per fruit was significantly higher in polyhouse with stingless bee pollinated crops (12.26 g) than in polyhouse with hand pollinated crops (8.61 g). Similarly, highest germination per cent was observed in seeds collected from the stingless bee pollinated crop (90.60) where as in hand pollinated treatment, it was recorded as 63.00 at 2 DAS (Plate 11). Higher yield was observed in bee pollinated crop (5.09 kg m^{-2}) compared to that of the hand pollinated crop (3.16 kg m^{-2}).

Thus stingless bee pollination had an added effect on the yield parameters *viz.*, per cent fruit set, single fruit weight, number of seeds per fruit, seed weight per fruit, germination percentage and yield with an increase in percentage of 40.74, 23.42, 63.15, 42.39, 43.80 and 61.00 respectively (Figure 6). The polyhouse with bee assisted pollination was economically feasible with a B:C ratio of 1.23 while that of the hand pollinated one, B:C ratio is 0.67 (Appendix I).

4.5 INCIDENCE OF PESTS AND DISEASES IN CUCUMBER

Although the protected cultivation are known to resist the entry of pest and disease invaders, due to UV preventing roof, insect proof side net, double door system and hygienic cultivation practices, pest and diseases occurred during the experiment. Incidence of aphids (Aphididae; Hemiptera) was observed 2 Months After Sowing (MAS). Incidence of downy mildew caused by *Pseudoperenospora cubensis* (Berk. & Curt.) Rostov. and powdery mildew caused by *Erysiphe cichoracearum* Jacz. were also recorded 2 MAS (Plate 12).

Table 9. Comparison of qualitative and quantitative yield parameters of bee pollinated and hand pollinated plot

Yield parameters	Bee assisted pollination	Hand pollination	t- value
*Fruit set (%)	76.00	54.00	2.24
*Malformed fruits (%)	16.10	14.00	NS
*Fruit length (cm)	26.07	25.99	NS
*Fruit diameter (cm)	7.18	6.81	NS
*Fruit weight (g)	685.00	555.00	2.29
*Number of seeds per fruit	344.10	210.90	2.67
*Seed weight per fruit (g)	12.26	8.61	2.13
*Germination (%)	90.60	63.00	7.58
T table value (0.05)	2.10		
Yield (kg m^{-2})	5.09	3.16	-

NS: No significant difference between both experiments

*Mean of 10 observations

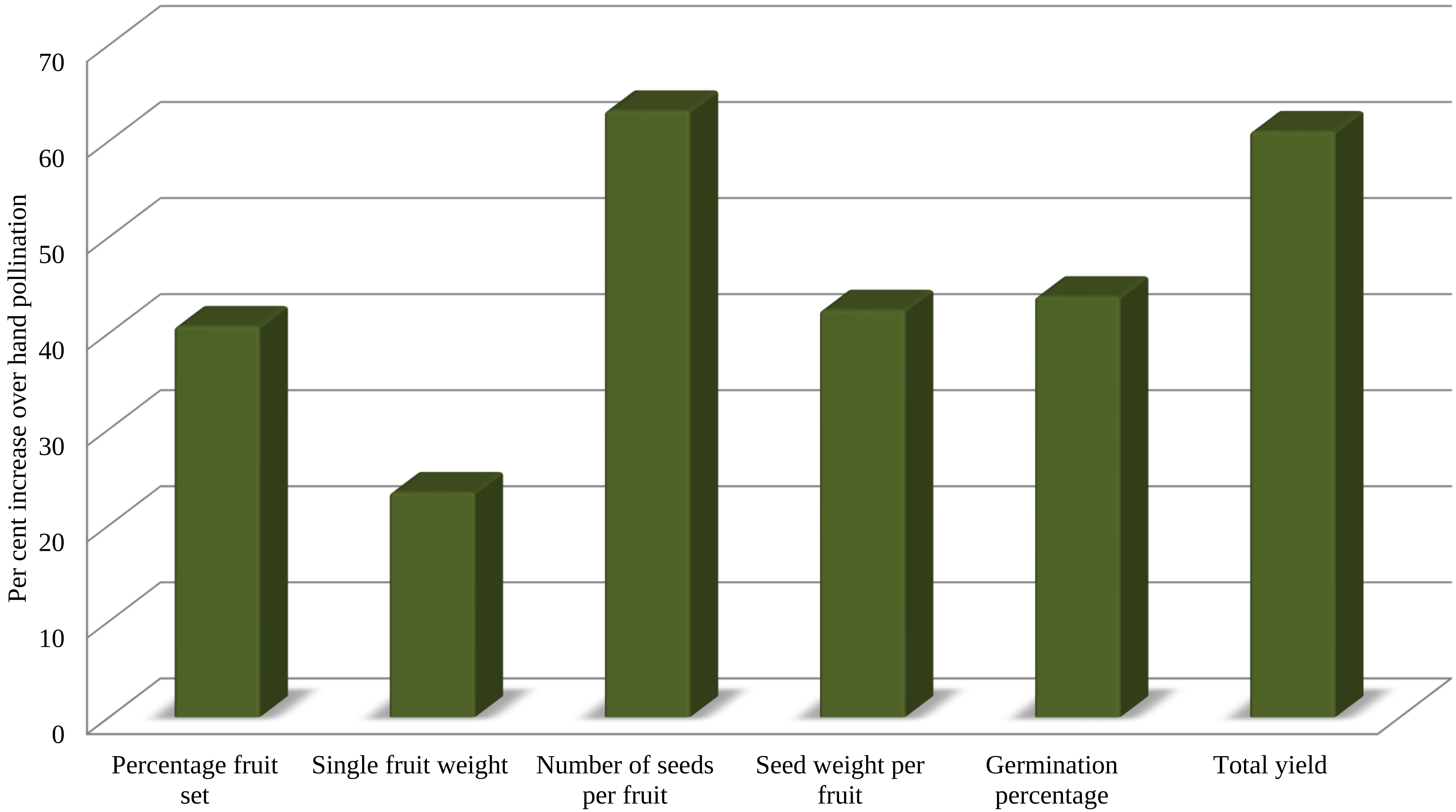
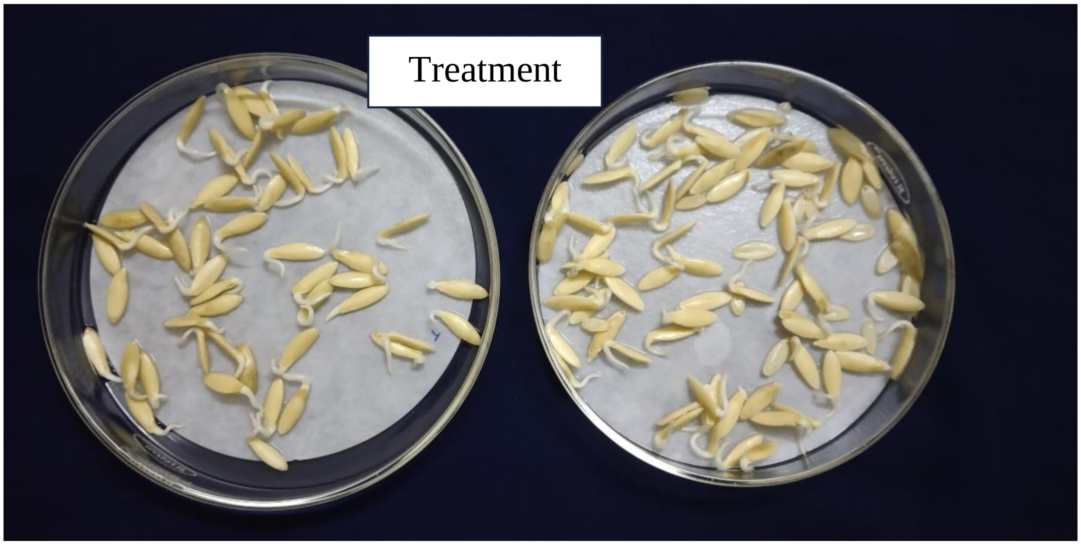


Figure 6. Per cent increase in yield parameters under stingless bee pollinated cucumber compared to control (hand pollination)



(A) Germination of seeds obtained from stingless bee pollination

(B) Germination of seeds obtained from hand pollination

Plate 11. Seed germination 2 DAS

(A) Downey mildew in cucumber

(B) Powdery mildew in cucumber

Plate 12. Incidence of diseases in polyhouse

Discussion

5. DISCUSSION

The present investigation on "Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) in cucumber (*Cucumis sativus* L.) under protected cultivation" was carried out from November, 2019 to February, 2020 at Department of Agricultural Entomology, College of Agriculture, Vellayani to evaluate the effect of pollination by stingless bee (*T. iridipennis*) on fruit set, yield and quality of salad cucumber under protected cultivation. The results obtained in the study are discussed in this chapter.

Stingless bees are highly diverse and abundant group of eusocial bees that inhabit the tropical and subtropical parts of the world. They are well known for medicinal value in their honey and pollen (Omar *et al.*, 2015; Yaacob *et al.*, 2018).

Meliponiculture is an art and science of keeping stingless bee in man-made nests, for their pollination services and hive products. It is being popularized because of their potential contribution in sustainable agriculture and for conserving biodiversity (Devanesan *et al.*, 2017; Karthick *et al.*, 2018).

Several species of stingless bees have been proven as good foragers and effective pollinators especially in enclosed conditions, indicating their potential as pollinators of different polyhouse crops (Slaa *et al.*, 2000). Stingless bee pollination has been found superior to open pollination (Malagodi-Braga and Kleinert, 2004), mechanical pollination and in tropical climates could be an alternative to the use of highly defensive African-derived *A. mellifera* or non-native bumble bees (*Bombus* spp.) (Cruz *et al*., 2005). Besides they are efficient than that of hand pollination (Nunes-Silva *et al*., 2013).

In India, *T. laeviceps* (Gadhiya, 2015) and *T. iridipennis* (Tej *et al.*, 2017) were the only examined stingless bees and were found to pollinate crops effectively inside enclosures. In the current investigation, *T. iridipennis* was used to pollinate non parthenocarpic cucumber grown in polyhouse

5.1 FORAGING BEHAVIOUR OF *T. iridipennis* IN POLYHOUSE

While recording the foraging behaviour, two different modes of alighting of *T. iridipennis* were observed in the present study. Most of the observed bees (78.00 %) were side workers which landed on the petals and proceeded towards the reproductive part (stamen or stigma). The rest of the foragers (22.00 %) were top workers, which landed directly on the top of stamen or stigma. Same bees were observed to perform both the modes of alighting on same flower during the same visit. In either ways they had their body dusted with adequate amount of sticky pollen grains. Even if a portion of pollen is loaded to corbicula, enough pollen will remain on their body hairs. This is transferred to the stigma unintentionally while they visit pistillate flowers seeking nectar.

This result confirms the earlier report of Bomfim *et al*. (2014) that regardless of the way of approach, pollen grains of watermelon flowers were transferred to the body of the stingless bees *Scaptotrigona* sp. Nov. Also, a large proportion of bee body, comes in contact with stigma of flowers due to their relativeiy small size and effectively pollinate the flowers.

The foraging activity of *T. iridipennis* foragers usually begins at 0750 h in the morning immediately after the anthesis of cucumber flowers. It was also observed that total duration of daily flight activity throughout the observation period (November 2019 to February 2020) was around 9 h. The observations were in accordance with that of Heard and Hendrikz (1993) who reported the length of the daily flight period of *T. carbonaria* ranging from 3 to 9 h during cooler months of the year.

The foraging rate was the highest (6.80 male flowers 5 min^{-1}) during the time of initiation of foraging activity (0800 – 0900 h) and was least (3.60 male flowers 5 min^{-1}) during the cessation time of foraging activity (1600-1700 h). But the foraging intensity was minimum during the initiation and cessation time of foraging activity (1.40, 1.20 and 0.80 bees male $flower^{-1}$ 10 min^{-1} recorded during 0800 – 0900, 1500 – 1600 and 1600 –

1700 h respectively). Maximum foraging intensity (4.40 bees male flower^{-1} 10 min^{-1}) was observed during 1000 – 1100 h.

Maximum time spent by pollen collectors per male flower was recorded as 44.20 sec during 1200 - 1300 h of the day. Similar observation was reported by Roopa (2002) where *T. iridipennis* spent maximum time during 1200 h in *Sechium edule* (Jacq.) under caged condition.

For nectar collection, stingless bee spent more time on female flower compared to that of male flowers. The visit to female flowers was longer during morning hours. Longest time spent by a single bee on female flower was recorded as 323.40 sec. The present findings are in line with Nicodemo *et al.* (2013), where the duration of visit in female flowers were longer than that of male flowers irrespective of the cucumber cultivar.

The foragers of *T. iridipennis* had their peak activity within the optimal period of stigma receptivity and pollen viability. The total number of incoming and outgoing foragers at the hive entrance increases with the ascent of the day reaches a maximum during 1300-1400 h (67.00 and 58.00 number of incoming and outgoing foragers respectively) and then decreases thereafter. This is in confirmation with the findings of Bomfim *et al.* (2016), where they found that the stigma of cucumber becomes most receptive during five to six hours after flower opening.

Cervancia and Bergonia (1991) reported that the pollinators were most active when nectar secretions were abundant. In cucumber, the quantity of nectar was the highest during 1200 h (Thakur and Rana, 2007; Satheesha, 2010). In the present study, the number of nectar foragers increases gradually along the day and reached a maximum during 1200 - 1400 h and then decreased thereafter throughout the three weeks of observations (Figure 7).

The study also revealed that the number of pollen foragers gradually increased at each time intervals up to 1000-1100 h followed by a slight decline during 1100-1200 h and then reached a highest peak at 1200-1300 h (during first and second week) or 1300-1400 h (third week) and declined thereafter (Figure 8). This may be because of the optimum climatic factors favorable for pollen collection during 1000-1100 h and following decline indicate the shifting of pollen collectors to nectar collectors. The second peak in number of pollen collectors may be due to the increase in total number of bees out for foraging. However, the number of nectar collectors was higher than that of pollen collectors.

The results are in close agreement with Roopa (2002) who reported that there were two activity peaks of *T. iridipennis* pollen foragers, first during 1000-1100 h and the second during 1400-1500 h in February. Santos *et al*. (2008) also reported two activity peaks for *N. testaceicornis* during 1000 and 1300 h.

The number of pollen grains collected by bees was high during morning hours. This corroborates with the results pertaining to foraging behaviour, highest foraging rate during 0800 – 0900 h, maximum foraging intensity during 1000 – 1100 h and maximum time spent by pollen collectors during 1200 - 1300 h. After midday the number of pollen grains collected was much less.

The present result is in accordance with the findings of Stanghellini *et al*. (2002) who reported that the pollen become scarce in flowers after midday. This is directly related to the intensity of flower visitation and pollen removal by insect visitors. In cucumber, more than 57 per cent of total available pollen is removed by pollinators within two hours after anthesis.

Besides, the study also revealed that the maximum bee activity was during maximum temperature (32.86 °C) and minimum relative humidity (59.80 %) (Figure 9). The result confirms with the earlier report of Jaapar *et al.* (2018) where they reported that the ideal temperature related to foraging behaviour for stingless bee *H. itama* was 29 ºC to 32 ºC.

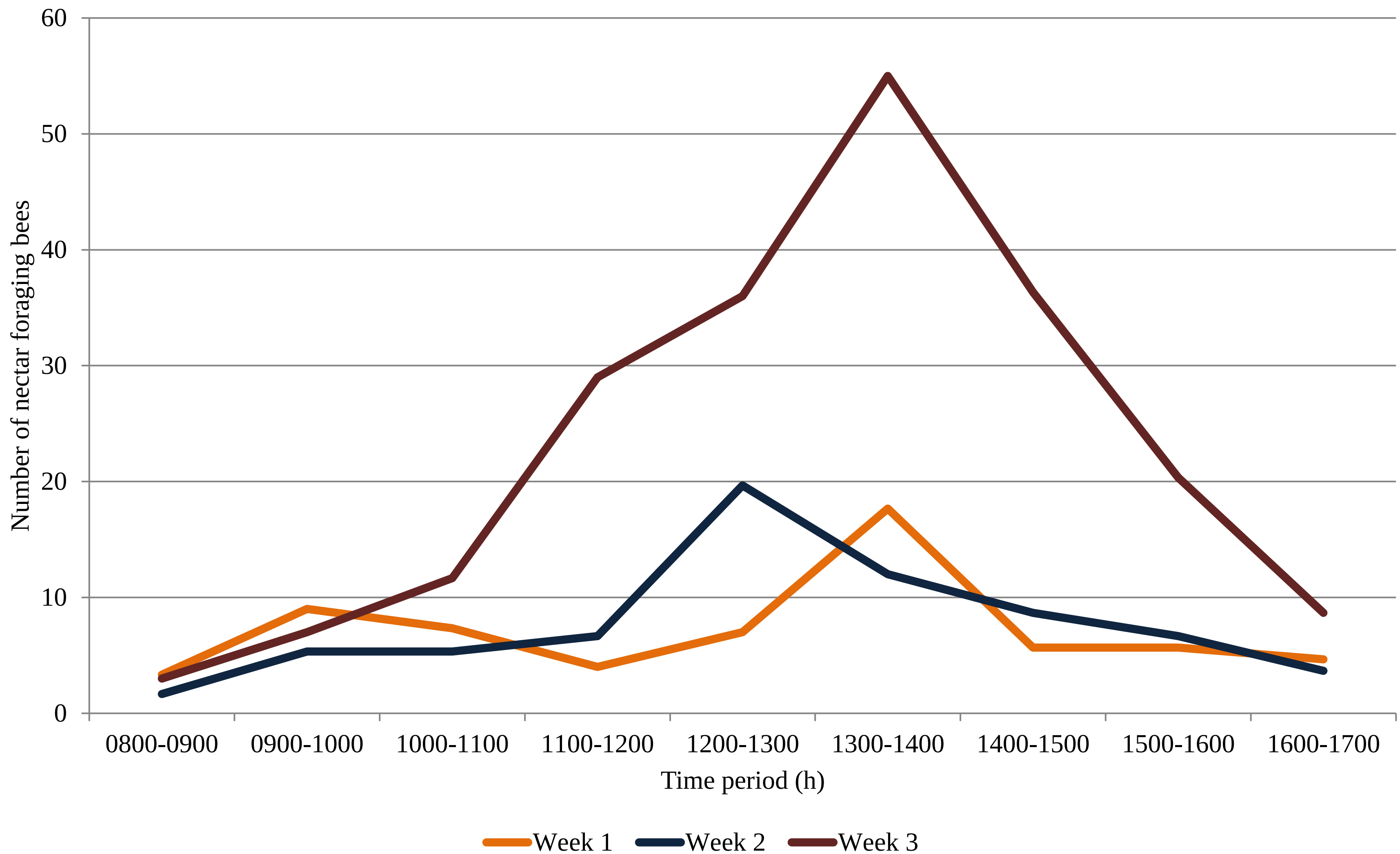


Figure 7. Diurnal variation in foraging activity of nectar collectors during different weeks

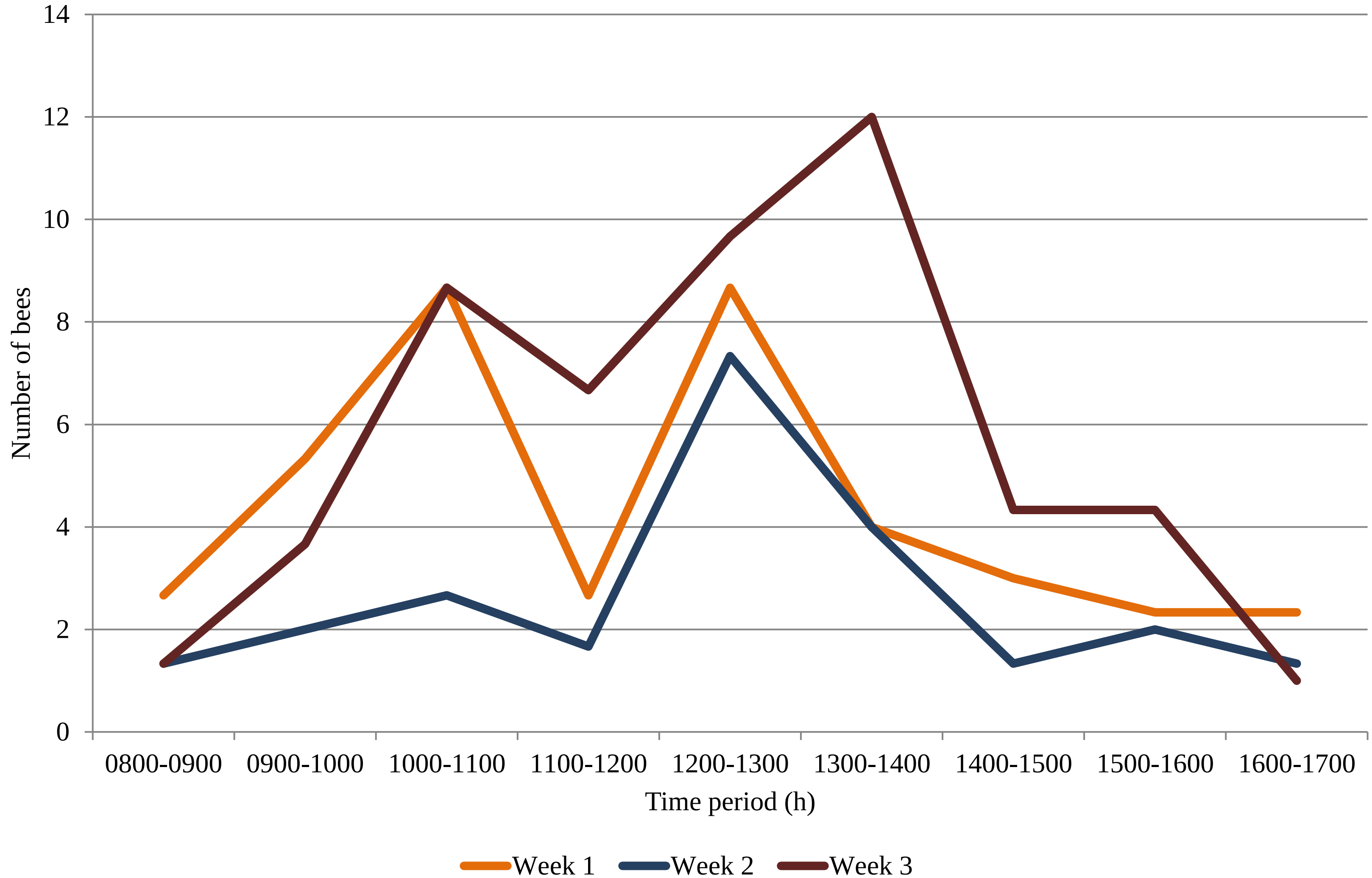


Figure 8. Diurnal variation in foraging activity of pollen collectors during different weeks

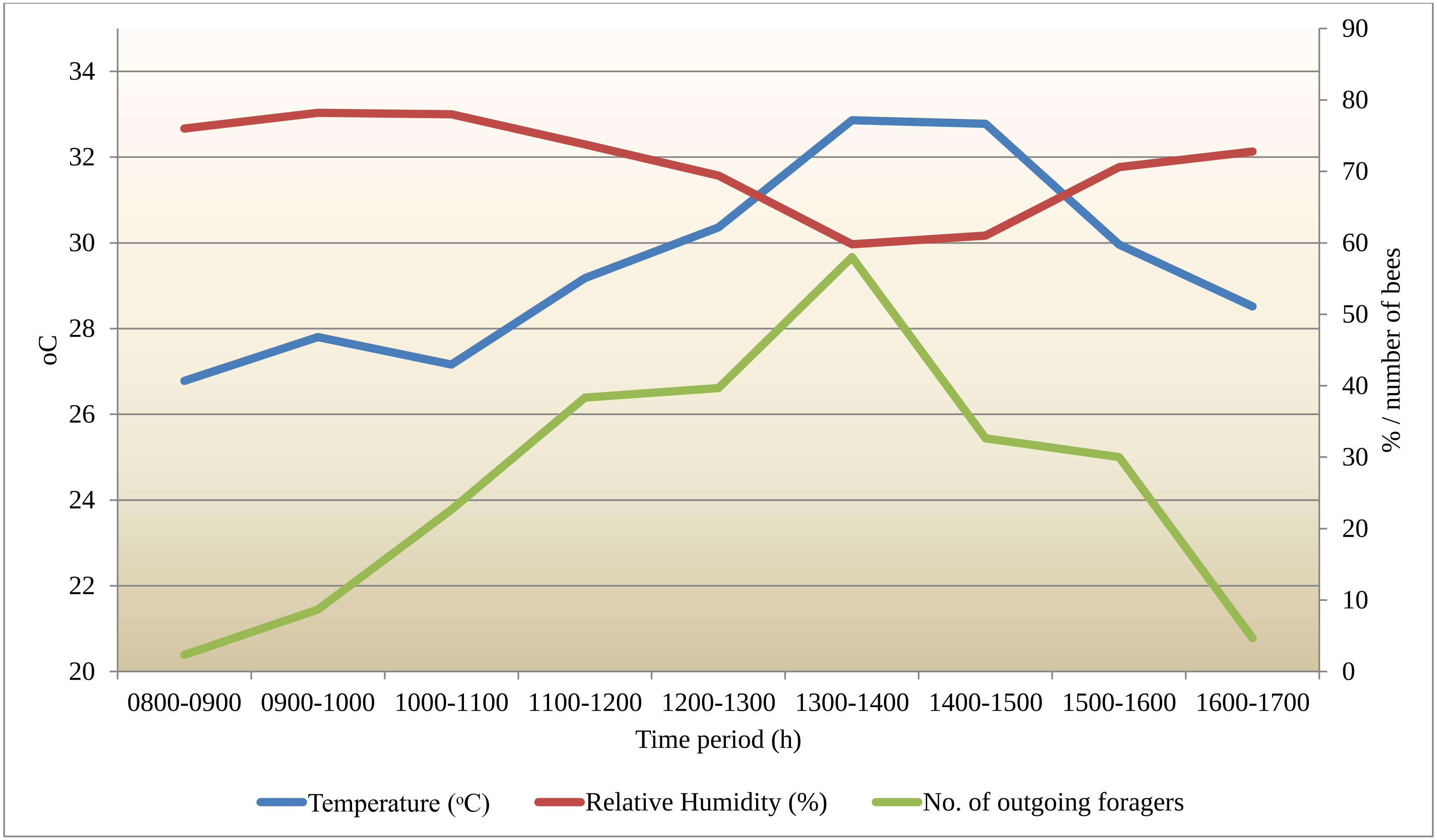


Figure 9. Influence of temperature and relative humidity on flight activity of *Tetragonula iridipennis*

There is a significant positive correlation between temperature and number of incoming foragers (r = 0.78) and a significant negative correlation between relative humidity and number of incoming foragers (r = -0.68). The influence of temperature accounted for 46 per cent variation in the number of incoming foragers (R^2 = 0.46) while the relative humidity accounted for 36 per cent variation in number of incoming foragers (R^2 = 0.36). The present inferences are comparable to the observations of Palma *et al.* (2008), where they recorded significant positive correlation of foraging activity (number of bees entering the hive and number of bees visiting the flowers) with temperature and negative correlation with relative humidity.

5.2 FLORAL BIOLOGY OF CUCUMBER

The ecology and floral biology reveals the entomophilous nature of cucumber plant. The flowers are bright yellow colour, bracteate, pedicellate, radially symmetrical, unisexual, pentamerus, epigynous, sepals 5, united, often deeply 5 lobed, petals 5, united to form a tube, often deeply 5 lobed and are produced in axes of leaves. Staminate flowers are generally born in clusters in leaf axils, while pistillate flowers are born singly in independent leaf axils and less frequent intervals than males. Staminate flowers usually appears 8 to 10 days prior to that of pistillate flowers. Also pollen grains are sticky.

This observation was in accordance with that of Mc Gregor (1976); Ruffner and Hall (1976) who reported that the plant and flower biology ensure the pollination requirement in cucumber.

The foraging activity of *T. iridipennis* synchronized with anthesis of cucumber flowers. The flowers opened from 0600 h to 0800 h in the morning. It appears that these bees remained active outside their nests only when the flowers were open. No foraging activity was observed after 1730 h during which the flowers remain closed. This will also agree with the findings of Cervancia and Bergonia (1991) who reported that the flower visitation of pollinators was in accordance with anthesis of cucumber flowers.

Pollen and nectar are usually considered as floral rewards for pollinating bees. For plants, the pollen deposition on stigma is the prerequisite of pollination, while for bees it is the essential food for the proper growth of their larvae. Accordingly, there should be a competition for pollen and the coevolution of plant and pollinators shows a mutual exploitation. Although pollination is happening, nectar serves an important role (Sakai, 1993; Westerkamp, 1996) which is the primary source of energy and water and is hidden at a greater depth within a flower (Nicolson *et al.*, 2007). The present study showed that the nectaries are bowl like structure observed at the base of the stamen in male flowers, whereas it surrounds the base of style forming a trough for accumulation of nectar in female flowers. This position enables the pollinators for easy access to the female nectar.

5.3 MORPHOLOGICAL ADAPTATIONS OF BEE IN RELATION TO FLORAL BIOLOGY

The proboscis of *T. iridipennis* was shorter (1.30 mm) compared to that of corolla tube length. The average length of corolla tube of male flowers was 6.14 mm and that of female flowers was of 5.23 mm. In order to reach the nectaries, bees have to insert their head into the corolla tube. According to evolutionary concept, nectar depth always has to exceed tongue (proboscis) length to maintain floral fitness in terms of fruit set (Nilsson, 1988). The deeper positioning of nectaries, not only prevent nectar desiccation but also forces bees to go deeper into the flower. This finally results in an exact positioning of pollen in stigmatic surface.

As the proboscis of stingless bee is much shorter than corolla tube of cucumber flowers, they insert their head inside the corolla, to reach nectaries. This could ensure the close contact of bee abdomen and hind legs with stigmatic lobes. Thus, the pollen grains adhering to the body hairs get deposited on stigma. Besides the nectar gland are able to refill the depleted nectar, which will attract more foragers and ensure enough pollen deposition (Westerkamp, 1996).

In the present study, average body length of bee is 3.69 mm, head length is 1.39 mm and width of the head is 1.74 mm. The gap between corolla tube and stamen tip was recorded as 1.35 mm while the gap between corolla tube and stigma tip was 1.91 mm. This gap is comparable with the head width of bees and the gap is wider in female flowers.

Willson and Agren (1989) reported that male flowers commonly offer more rewards (nectar and pollen) than female flowers (nectar). Although female flowers of many species offer more nectar than male. According to Nicodemo *et al*. (2007) the nectar quantity produced on female flowers was 138.90 per cent higher than that of male flowers in *Cucurbita maxima* Duchesne. Satheesha (2010) also reported that the quantity of nectar produced by female flowers of cucumber per day (16.10 µL) was more than that of male flowers per day (13.23 µL).

These studies support the present investigation that, even if female flowers are less produced compared to that of male flowers, their preference to pollinators is not less. This may be because of the higher quantity of nectar produced by females. Also female flowers have shallow corolla tube and wider gap between corolla wall and stigma tip compared to male flowers. The head width of bees (1.74 mm) is smaller than the gap between corolla wall and stigma tip (1.91 mm) and is perfectly fit for *T. iridipennis* foragers to reach nectaries at the base of cucumber flowers.

5.4 EFFICIENCY OF STINGLESS BEE POLLINATION ON CROP YIELD

Upon analyzing the yield parameters of cucumber, it was revealed that there is a significant increase in fruit set in stingless bee pollinated crop (76.00 %) compared to hand pollinated (54.00 %). The present result is in line with the findings of Nicodemo *et al.* (2013), who reported an increase in fruit set of cucumber by stingless bee pollination. It was also observed that there is no significant difference in per cent malformed fruits in either polyhouses with stingless bee and hand pollinated ones.

According to Kremen *et al*. (2004) pollen deposition is strongly related to fruit production. Adequate pollination is essential for development of uniform fruits and viable seeds (Abrol, 2015). Deformed fruits are the result of unfertilized ovules. This is due to the uneven distribution of pollen grains over stigmatic surface, absence of pollen deposition on any of the stigmatic lobe may be due to the insufficient pollen grains deposited. Bomfim *et al*. (2014) reported that repeated visit by stingless bee is needed for development of uniform fruits in watermelon.

No significant variation in yield parameters like length and diameter of fruit was observed between the two treatments. Unlike the present study, Tej *et al.* (2017) recorded a significant increase in fruit length (8.50% and 5.70 % increase) for parthenocarpic cucumber pollinated by stingless bee compared to respective control. The fruit girth was also significantly higher (6.50% and 5.60 % increase) for stingless bee pollinated parthenocarpic cucumber compared to respective control. However, in the present study, the location of polyhouse and the variation in microclimate inside also influence the foraging behaviour and in turn the yield attributes.

The results synchronize with the findings of Greco *et al.* (2011) who reported that fruit length and fruit size was little affected by bee pollination. Also, Gadhiya (2015) reported that there was no significant difference observed in quality of fruit between hand pollinated and stingless bee pollinated musk melon.

The yield parameters of cucumber *viz.*, single fruit weight, number of seeds per fruit, seed weight per fruit and germination per cent (2 DAS) was significantly higher for stingless bee pollinated crop (685.00 g, 344.10 seeds fruit^{-1}, 12.26 g and 90.60 %, respectively) compared to hand pollinated one (555.00 g, 210.90 seeds fruit^{-1}, 8.61 g and 63.00 %, respectively). The observations were in accordance with that of Cauich *et al.* (2006) who reported an increased seed production in green house grown *C. chinense* by stingless bees. dos Santos *et al*. (2009) also reported that stingless bee pollination effectively increased the fruit weight and number of seeds of greenhouse grown tomato.

Higher yield was recorded in crop subjected to augmented pollination (5.09 kg m^{-2}) than that of the hand pollinated one (3.16 kg m^{-2}). Cervancia and Bergonia (1991) reported an increased quality seed count in bee pollinated plants and suggested the potential of introducing bee colonies to increase seed yield. According to Delaplane and Mayer (2000), more seeds will develop when large numbers of pollen grains are transferred. Seeds in turn stimulate the development of surrounding ovary tissue so as to form larger fruits. In this way pollination improves both the yield as well as the size of the fruits.

5.5 INCIDENCE OF PESTS AND DISEASES IN CUCUMBER

Incidence of downy mildew and powdery mildew was observed 2 months after sowing the cucumber plant. According to Reshma (2016), downy mildew is associated with cucumber grown under protected environment, irrespective of the season. Wahul (2016) reported powdery mildew as one of the predominant diseases in cucumber grown under protected environment and occur year round.

In the present study, the incidence of aphids (Aphididae: Hemiptera) was observed during reproductive stage of the crop (2 MAS). Thamilarasi (2016) also reported the incidence of aphid in cucumber grown in polyhouse during 45 days after sowing.

Summary

6. SUMMARY

The present study entitled "Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) in cucumber (*Cucumis sativus* L.) under protected cultivation" was conducted in the polyhouse at College of Agriculture, Vellayani from November 2019 to February 2020. The objective was to evaluate the effect of pollination by stingless bee (*T. iridipennis*) on fruit set, yield and quality of salad cucumber under protected cultivation.

The foraging behaviour of *T. iridipennis* inside the protected environment was observed to evaluate their diurnal variations in resource collection and pollination ability in cucumber flowers. The observations on mode of alighting of bees, foraging rate, foraging intensity, foraging speed, number of incoming and outgoing bees were recorded at hourly intervals starting from 0800-0900 h to 1600-1700 h for 5 minutes. Data on temperature (°C) and relative humidity (%) were also recorded using a data logger placed inside the polyhouse.

The foragers were observed to visit the cucumber flowers from 0750 h in the morning, just after the anthesis (0600 to 0800 h) of cucumber flowers and continued till 1730 h in the evening, extend to a total of nine hours of foraging. Most of the observed bees (78.00 % were side workers) land on the petals and proceed towards the reproductive part (stamen or stigma). The rest of the foragers (22.00 % were top workers), land directly on the top of stamen or stigma. Same bees were observed to perform both the modes of alighting on same flower during the same visit.

Observations on foraging rate, revealed that an individual forager can visit highest mean of 6.80 male flowers 5 min^{-1} during the time of initiation of foraging (0800 – 0900 h) and the least recorded was a mean of 3.60 male flowers 5 min^{-1} during the cessation time of foraging activity (1600-1700 h). Maximum foraging intensity was observed with a highest mean of 4.40 bees male $flower^{-1}$ 10 min^{-1} during 1000 – 1100 h. Maximum time spent by pollen collectors per male flower was recorded as 44.20 sec during 1200 - 1300 h of the day. Also the number of pollen grains collected by bees was high during morning hours than in the evening.

The pollen and nectar gatherers were distinguished by the presence and absence of pollen load at their respective corbicula. The time spent by nectar collectors on male and female flowers varies significantly along the day with a peak of 141.20 sec (0800 – 0900 h) and 323.40 sec (1200 – 1300 h) on male and female flowers respectively. Irrespective of the time period stingless bee spent more time on female flower compared to that of male flowers in respective time intervals.

The total number of incoming and outgoing foragers at the hive entrance increases with the ascent of the day reaches a maximum during 1300-1400 h (67.00 and 58.00 number of incoming and outgoing foragers respectively) and then decreases thereafter.

The number of foragers returning to the hive with either pollen or nectar was counted from 0800 to 1700 h at hourly intervals for 3 weeks, and the foraging behavior was evaluated. The results revealed that the number of pollen foragers increases each time intervals up to 1000-1100 h followed by a slight decrease during 1100-1200 h, and then reaching a highest peak at 1200-1300 h (during first and second week) or 1300-1400 h (third week) and declines further. The number of nectar foragers increases gradually along the day and reach a maximum during 1200-1300 h (second week) or 1300-1400 h (third week) and then decreases.

There is a significant positive correlation between temperature and number of incoming foragers (r = 0.78) and a significant negative correlation between relative humidity and number of incoming foragers (r = -0.68).

The flower biology of *C. sativus* were studied to assess the relationship of plant and pollinator. The flowers are entomophilic in nature with bright yellow colour, bracteate, pedicellate, radially symmetrical, unisexual, pentamerus, epigynous, sepals 5, united, often deeply 5 lobed, petals 5, united to form a tube, often deeply 5 lobed and are produced in independent axes of leaves. The male flower bud appeared (25 DAS) an average of 8 days prior that of female flower bud (34 DAS). The staminate and pistillate flowers were bloomed 31 DAS and 39 DAS respectively. The mean size of staminate and pistillate flowers was 4.35 cm and 4.63 cm diameter respectively.

Studies on the floral biology of *C. sativus* revealed that male flowers have 5 stamens with twisted anthers having a mean length of 5.02 mm. Female flowers have 6 stigmas, with an average 2.97 mm in length. The length of style is 3.15 mm and that of the tricarpellary long inferior ovary is 3.30 cm in length. The nectaries are present at the base of the stamen which is a bowl like structure in male flowers, whereas it surrounds the base of style forming a trough for accumulation of nectar in female flowers. This nectar position helps the pollinators to easily access female nectar.

Observations on floral biology of cucumber and morphology of stingless bee were recorded to assess the adaptation of bees in relation to flower biology. Individual bees (nectar foragers) collect nectar by inserting their head into the corolla tube of both male and female flowers to reach the nectaries. The average length of corolla tube of male flowers was 6.14 mm and that of female flowers was of 5.23 mm. The gap between corolla tube and stamen tip was recorded as 1.35 mm while the gap between corolla tube and stigma tip was 1.91 mm.

The average body length of stingless bee is 3.69 mm. Average length of head is 1.39 mm whereas the width of the head is 1.74 mm. The length of proboscis ranged from 1.19 to 1.37 mm with an average of 1.30 mm.

Observations on yield parameters of cucumber revealed that there is a significant increase in per cent fruit set in stingless bee pollinated crop (76.00 %) compared to hand pollinated (54.00 %). No significant variation in parameters like per cent malformed fruit, length and diameter of fruit was observed between the two treatments.

The yield parameters of cucumber *viz.*, single fruit weight, number of seeds per fruit, seed weight per fruit and germination per cent (2 DAS) was significantly higher for stingless bee pollinated crop (685.00 g, 344.10 seeds fruit^{-1}, 12.26 g and 90.60 %

respectively) compared to hand pollinated one (555.00 g, 210.90 seeds fruit^{-1}, 8.61 g and 63.00 %, respectively). Higher yield was recorded in crop subjected to augmented pollination (5.09 kg m^{-2}) than that of the hand pollinated one (3.16 kg m^{-2}). Thus, stingless bee pollination has a prominent effect on the yield parameters of crop *viz.*, single fruit weight, number of seeds per fruit, seed weight per fruit, germination percentage and yield with an increase in percentage of 23.42, 63.15, 42.39, 43.80 and 61.00, respectively.

The polyhouse with bee assisted pollination was economically feasible with B:C ratio 1.23 and polyhouse without bee (hand pollination) with B:C ratio 0.67.

The present study reveals the highest foraging rate as 6.80 male flowers 5min^{-1} during 0800-0900 h, highest foraging intensity as 4.40 bees male flower^{-1} 10 min^{-1} during 1000 – 1100 h and longest time spent by bee per male flower as 44.30 sec during 1200-1300 h. The nectar and pollen collection peaks during 1200-1400 h. The stingless bee effectively increase the yield parameters *viz.*, per cent fruit set, single fruit weight, number of seeds per fruit, seed weight per fruit and germination percentage, while they did not affect other parameters. Sixty one per cent yield increase was recorded in stingless bee pollinated salad cucumber when compared to the hand pollinated one under protected condition.

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POLLINATION EFFICIENCY OF STINGLESS BEE, *Tetragonula iridipennis* (Smith) IN CUCUMBER (*Cucumis sativus* L.) UNDER PROTECTED CULTIVATION

by

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ABSTRACT

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ABSTRACT

The present investigation entitled "Pollination efficiency of stingless bee, *Tetragonula iridipennis* (Smith) in cucumber (*Cucumis sativus* L.) under protected cultivation" was conducted in the polyhouse at College of Agriculture, Vellayani from November 2019 to February 2020. The objective was to evaluate the effect of pollination by stingless bee (*T. iridipennis*) on fruit set, yield and quality of salad cucumber under protected cultivation.

The foraging behaviour of *T. iridipennis* inside the protected environment was observed to evaluate their diurnal variations in resource collection and pollination ability in cucumber flowers. Observations on mode of alighting of bees, foraging behaviour of stingless bees, floral biology of *C. sativus* and pollination efficiency of stingless bees in terms of the quantitative and qualitative parameters were recorded.

Most of the observed bees were side workers (78 %), which landed on the petals and proceeded towards the reproductive part (stamen or stigma) while the rest of the foragers (22 %) were top workers, which landed directly on the top of stamen or stigma. Foraging rate was the highest (6.80 male flowers 5 min^{-1}) during the time of initiation of foraging (0800 – 0900 h), while maximum foraging intensity was recorded as 4.40 bees male $flower^{-1}$ 10 min^{-1} during 1000 – 1100 h. Maximum time spent by pollen collectors per male flower was recorded as 44.20 sec. during 1200 - 1300 h of the day during which maximum pollen grains were observed from the body of the bees.

Irrespective of the time period, stingless bee spent significantly more time on female flowers compared to that of male flowers in respective time intervals.

The diurnal activity of a colony assessed in terms of the total number of incoming and outgoing foragers at the hive entrance revealed that their number increased with the ascent of the day, reached a maximum during 1300-1400 h (67.00 and 58.00 number of incoming and outgoing foragers respectively) and then decreased thereafter. Weekly

observations showed that the pollen and nectar foraging activity of stingless bees varies in a day with the peak period during 1200-1400 h.

A significant positive correlation was recorded between temperature and number of incoming foragers (r = 0.78) while a significant negative correlation was observed between relative humidity and number of incoming foragers (r = -0.68).

Studies on the floral biology as well as the morphology of bees has revealed that the individual nectar foraging bee insert its head into the corolla tube of both male and female flowers to reach the nectaries.

Significant increase in per cent fruit set was observed in stingless bee pollinated crop (76%) compared to hand pollinated one (54%) whereas the qualitative yield parameters like per cent malformed fruit, length and diameter of fruit were statistically on par. With regard to the quantitative yield parameters significantly higher single fruit weight, number of seeds per fruit and germination per cent (2 DAS) (685.00 g, 344.10 seeds/ fruit and 90.60 %, respectively) was recorded from the stingless bee pollinated crop than that of the control crop (555.00 g, 210.90 seeds/ fruit and 63.00 %, respectively). Higher yield was observed in augmented pollination (5.09 kg m^{-2}) compared to hand pollination (3.16 kg m^{-2}). Apart from this, bee assisted pollination was economically feasible with better B:C ratio (1.23) than that of the hand pollinated one (0.67).

The foraging activity of bees varied in a day with the peak period during 1200-1400 h. Stingless bee pollination had a profound influence on the per cent fruit set as well as the quantitative parameters like fruit weight, germination percentage and number of seeds per fruit. Augmentative pollination with stingless bee under protected cultivation resulted in increased yield of 61 per cent in *C. sativus*.

Appendix

APPENDIX I

Benefit - cost ratio

Particulars	Bee assisted pollination	Hand pollination
Expenditure (Rs.)		
Cost of planting material-seeds	50	50
Cost of FYM	500	500
Cost of Vermicompost	200	200
Cost of net for trailing	1,250	1,250
Labour charge	4,800	4,800
Cost of stingless bee colony	2,000	-
Additional labour charge for hand pollination	-	3,200
Maintenance cost for polyhouse (water, electricity and others)	500	500
Total cost of production (Rs.)	9,300/-	10,500/-
Returns		
Total Nos. of harvest	5	5
Total yield (kg)	382	237
Total returns (Rs.)	11,460/-	7,110/-
B:C Ratio	1.23	0.67
Percentage increase in yield over control : 61%		