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Plant-pollinator interaction, pollinator diversity and relative abundance in bottle gourd (*Lageneria siceraria*) in Coimbatore

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Abstract
Insect pollinators are essential not only for enhanced food production but also play a major role in improving the quality produces. Various threatening agricultural practices decline the pollinators and the service they provide. The current study was designed to record different insect pollinators, their abundance and plant-pollinator interaction in bottle gourd (*Lageneria siceraria*) during 2022 at Coimbatore region. Based on the current research, nine species were recorded as floral visitors/pollinators in bottle gourd. Out of the nine species, two species belonged to Hymenoptera, two to Coleoptera, three to Lepidoptera and one to Hemiptera. Major pollinators/ floral visitors were evaluated for their abundance and pollinating efficiency. *Haptonchus* sp. (Nitidulidae: Coleoptera) (72.04%) was the predominant species recorded, followed by *Nesidiocoris* sp. (Miridae: Hemiptera) (22.61%) and *Apis cerana indica* Fab. (Apidae: Hymenoptera) (2.03%). Abundance of Hymenopterans (*A. cerana indica* and *Halictus* sp.) was less when compared to *Haptonchus* sp. But the pollen-pollinator interaction of hymenopterans was quantitatively higher than insects of other orders irrespective their abundance. Palynological studies play an essential role in determining the plant-pollinator interaction/ pollen pollinator interaction, pollen taxonomy and the floral source of honey.

Keywords: Bottle gourd, pollinator diversity, plant-pollinator interaction, palynology

Introduction
Cucurbitaceae is one of the important families of vegetable crops. This family consists of many vegetables collectively called as cucurbit [4]. Cucurbits are otherwise called as Gourds or Melons. There are more than 118 genera and 825 species under this family which are widely cultivated throughout the parts of tropical regions [5]. Cucurbitaceae is an economically important family with a number of domesticated species. Among the domesticated species *Lageneria siceraria* is a peculiar one with hard shelled and bottle shaped variants. Fruits are large, varies in shapes like bottle, cylindrical, flask-shaped or globose [8]. Bottle gourd is also known as Calabash or White flower gourd which can be grown in both rainy and summer seasons. The name of this gourd is derived from its fruit shape. Bottle gourd is a monoecious (male and female structures does not occur in same flower) creeping vine with white flowers opening at dusk and closes at next day morning. From the time of anthesis pollen remains viable until the next morning. The sticky and slimy nature of pollen restricts the movement of pollen from anther of one flower to stigma of another flower. Most of the cucurbits come under cross pollinated group because of their monoecious nature and mainly depend on various pollinating agents for their pollination. In bottle gourd, insects play an important role in pollination [6]. In recent days pollinators have declined due to various reasons such as change in farm land use, intense use of pesticides, diseases, environmental pollution, climate change etc., [3]. To reduce the declining rate and to conserve pollinators, information about their diversity and abundance are essential. Hence the studies have been undertaken to record different insect pollinators and their abundance in bottle gourd. Palynology is the scientific study of pollen grains and the determination of their origin, which plays a crucial role for understanding the mechanism of plant-pollinator interactions. Pollen from various flowers is distinguished by its form, shape, size, and ornamentation. Microscopical study of pollen from plants that are pollinated by insect visitors is a well-established approach for confirming the diversity of pollinators and to determine the source of honey in a given location. Pollen study has significant application in recognition of insect visitors that helps in pollination.

Materials and Methods

The present study on plant-pollinator interaction in bottle gourd, pollinator diversity and abundance was conducted at a Farmer's holding in Thondamuthur (10.998689º N and 76.801541º E) in Coimbatore district, Tamil Nadu, India during 2022. The crop was sown in an area of half acre in January 2022 and flowering started at 45 days after sowing. Diversity and abundance study was started at 50 per cent blooming stage of the crop.

Diversity of floral visitors/pollinators

To record the pollinator diversity on bottle gourd, sweeping net method was used. Insects were collected using sweep net during peak blooming period of the crop. Soft bodied and minute insects were collected using camel hair brush from stem and branches of the plant. The collected specimens were preserved as dry specimens for later identification. Minute insects were preserved in 70% ethanol in vials and stored.

Plant-pollinator interaction

To study the plant-pollinator interaction, pollen grains were collected from different insect visitors and morphologically analyzed and confirmed using standard samples of pollen collected from bottle gourd plant. The mature male flowers carrying polleniferous materials were collected during the time of anthesis from experimental farm. Fully bloomed flowers were selected and picked individually and the samples were preserved individually in butterpaper covers for further morphological studies. Floral visitors were collected using sweep nets and forceps (for minute insect visitors) from the experimented field. The collected floral visitors were placed in sterile water in eppendorf tubes to reveal the presence and identity of the pollen.

Pollination efficiency index

Loose pollen grains were measured by employing a sweep net to gather foraging floral visitors in the field during peak foraging hours. The insect visitors are placed in a glass vial containing 70% alcohol. The vials were then vigorously shaken to unload pollen grains out of their bodies. Volume was made up to 5 ml. An aliquot of 0.01ml was collected then examined by placing in a haemocytometer and observed under a stereozoom microscope.

Pollination efficiency index =
Performance score of abundance * Performance score of loose pollen grains adhering on the body of floral visitors.

$$\text{PS of Loose Pollen} = \frac{\text{LP}i}{\sum_{i=1}^{n} LPi}$$

$$\text{PS of Pollinator Abundance} = \frac{\text{PA}i}{\sum_{i=1}^{n} PAi}$$

Morphological characterization of pollen grains

Collected samples were dusted on slide to delineate its shape, size and ornamentation. To enhance the structure of pollen grains, glycerine was used at the time of mounting. The prepared slides were examined through research microscope. For the accuracy of the results, ten pollen grains from the specimen were randomly analysed and measured. Pollen grains were observed under different magnifications in phase contrast microscope and scanning electron microscope at the Department of Nanotechnology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Abundance of floral visitors/pollinators

To measure the abundance of different floral visitors, five randomly selected one metre square area were fixed and the observations (no. of floral visitors visited) were recorded for five minutes in the specified fixed area at an hourly interval from 6.00-18.00 hrs [2]. The data was separated time wise and insect order wise then documented for further analysis.

Results and Discussion

Diversity of floral visitors/ pollinators in bottle gourd

A bottle gourd flower begins to open at late evening (after 17.00 Hrs) and remains in an opened state from 8.00-20.00 Hrs after flowering. Male flower blooms and closes earlier than female flower [9]. In bottle gourd, flower normally closes between 10.00 and 12.00 Hrs. The present investigation was carried out throughout the day due to constant association of some floral visitors with flowers even after it closes.

The bottle gourd blooms were visited by nine species of floral visitors from eight families belonging to four orders in this study. Among the floral visitors observed, Lepidopterans had rich diversity with 3 species of moth and 1 species of butterfly belonging to three different families which was followed by Coleopterans and Hymenopterans with two species each. One species of Hemiptera was also recorded as floral visitor of bottle gourd. Morimoto (2004) [6] and Subhakar (2015) [13] earlier revealed the diversity and richness of Lepidoptera in bottle gourd blossoms.

Lepidopteran flower visitors consist of 3 species, *Hippotion celerio* L. from Sphingidae, *Diaphania indica* S. from Crambidae and *Pachliopta hector* L. from Papilionidae. Coleopterans included two species, one from each of the families Chrysomelidae (*Aulacophora foevicollis* Lucas) and Nitidulidae (*Haptonchus* sp.). Two species of Hymenoptera were found, one from the Apidae family (*A. cerana indica*) and one from the Halictidae family (*Halictus* sp.). Hemiptera consists of one species from family Miridae (*Nesidiocoris* sp.). The findings of this study are nearly similar to Padhiyar (2021) [7].

Srikanth (2013) [12] reported more than 20 species of floral visitors in bottle gourd. The reason for more number of pollinator species visiting the crop may be due to the use of attractants (Citral a and citral b) to attract pollinators. *Xylocopa fenestrata* F., visited bottle gourd flowers for pollen and nectar and performed as a good pollinator, according to Sihag (1990) [11]. Shrivastava (1990) [9] reported *Cyrtopeltis tenuis* and *Arthoscista hilarialis* W. as bottle gourd pollinators. *Xylocopa* sp. was not found on bottle gourd in Coimbatore, according to the current findings. Subhakar (2015) [13] also reported diverse Lepidopteran pollinators in their study but most of those pollinators were not observed in the present investigation. The modest differences between the current study and the previous studies on floral visitors to bottle gourd could be attributable to the floral reward, floral anthesis time and local meteorological circumstances.

Table 1: List of floral visitors of Bottle gourd

S. No.	Floral Visitors	Family	Order
1.	*Apis cerana indica* Fab	Apidae	Hymenoptera
2.	*Halictus* sp.	Halictidae	Hymenoptera
3.	*Aulacophora foevicollis* L.	Chrysomelidae	Coleoptera
4.	*Haptonchus* sp.	Nitidulidae	Coleoptera
5.	*Nesidiocoris* sp.	Miridae	Hemiptera
6.	*Hippotion celerio* L.	Sphingidae	Lepidoptera
7.	*Diaphania indica* S.	Crambidae	Lepidoptera
8.	*Pachliopta hector* L**.**	Papilionidae	Lepidoptera

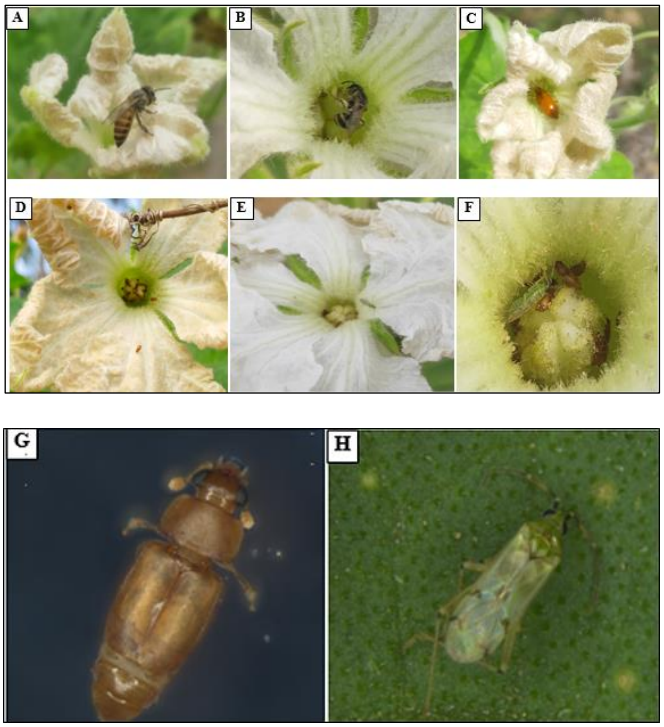

Fig 1: Floral visitors of bottle gourd (a - h)
a) *A. cerana indica*; b) *Halictus* sp.; c) *A. foevicollis*; d) *Haptonchus* sp.; e) *Nesidiocoris* sp.; f) *Haptonchus* sp. and *Nesidiocoris* sp. forage within the flower g) LEICA image of *Haptonchus* sp.; h) LEICA image of *Nesidiocoris* sp.

Plant-pollinator interaction

Plant samples showed that bottle gourd pollens are isopolar in shape with three pores and three colpi. Compound type aperture was observed. The exine was reticulate and had minute perforations. The mean size of the pollen grain was found to be 51.80 µm. The pollen grains separated from the pollinators except lepidopterans were morphologically analysed and compared with the pollen characters derived from the plant sample.

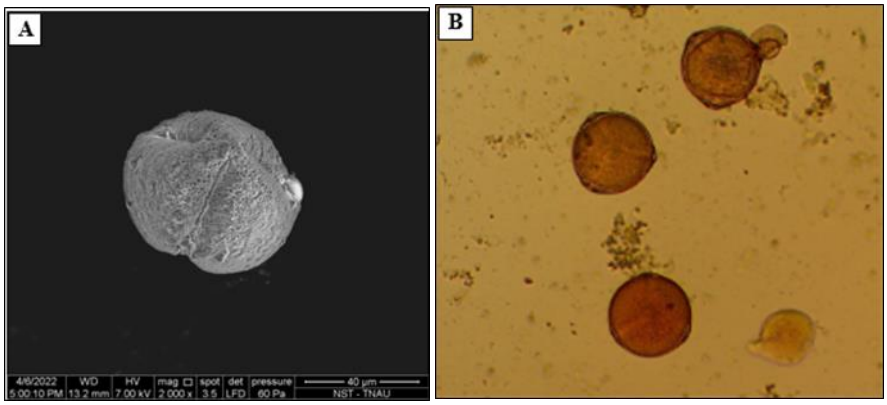

Fig 2: a) SEM image of bottle gourd pollen b) Phase contrast image of bottle gourd pollen

Pollen grains separated from *A. cerana indica*, *Halictus* sp., *A. foevicollis*, *Haptonchus* sp. and *Nesidiocoris* sp. were nearly similar to the key characters of bottle gourd pollen grains. But the beetle-flower association in related to pollination yet to be clarified by in-depth studies with special reference to certain species. The pollinating efficiency of Hymenopterans relatively higher than other pollinators. Pollination efficiency index of *A.cerana indica* was found to be 111.46 (Table 2).

Table 2: Pollination efficiency index different floral visitors

Floral visitors	Abundance of floral visitors		Number of loose pollen grains sticking on the body of floral visitors		Pollinating efficiency index	Rank
	PA	PS	PL	PS		
A. cerana indica	0.45	2.09	67200	53.33	111.46	1
Halictus sp.	0.04	0.19	58600	46.50	8.64	2
Haptonchus sp.	15.88	73.76	94	0.07	5.50	3
Nesidiocoris sp.	4.98	23.13	54	0.04	0.99	4
A. foevicollis	0.18	0.84	65	0.05	0.04	5
Total	21.53	100.00	126013	100		

Abundance of floral visitors/ pollinators in bottle gourd

Observations recorded on different floral visitors/ pollinators were given in Table 3. The table shows the data collected on the activities of different flower visitors at different times of the day in a one metre square area over a five-minute period. According to the observations *Haptonchus* species was predominant floral visitor with 72.04% of relative abundance followed by *Nesidiocoris* sp. (22.61%)**,** *A. cerana indica* (2.03%) and *Pachliopta hector* (1.65%).

Aside from these, *A. foevicollis* and *Hippotian celerio* were shown to have an activity accounting for about 0.83% and 0.47%. *Halictus* sp. and *Diaphania indica* were found to be least active with only 0.20% and 0.17% of abundance. This current investigation on abundance of floral visitors is in line with Padhiyar *et al*., (2021) [7] who reported that the *Nesidiocoris* sp. and *Haptonchus* sp. was abundant floral visitors in bottle gourd. Srikanth (2013) [12] identified Hymenopterans as major floral visitors of bottle gourd. According to Subhakar and Sreedevi (2011) [13], *Diaphania indica* (28.43 percent) had the highest proportion of bottle gourd pollinators, followed by *H. celerio* (25.73%) and *Arthoscista hilarialis* (24.65%).

Table 3: Population of insect visitors on different times of the day in bottle gourd

Floral visitors	Number of floral visitors/m²/5 minutes at different day hours												
	06-07	07-08	08-09	09-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	Mean
Apis cerana indica	0.68 (1.05)^{c}	0.86 (1.12)^{c}	1.00 (1.22)^{c}	0.82 (1.13)^{c}	0.88 (1.16)^{c}	0.10 (0.77)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{c}	0.52 (0.98)	0.50 (0.95)^{d}	0.45 (0.93)
Halictus sp.	0.00 (0.71)^{e}	0.04 (0.73)^{e}	0.14 (0.80)^{f}	0.14 (0.80)^{f}	0.10 (0.77)^{e}	0.08 (0.76)^{d}	0.02 (0.72)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{c}	0.00 (0.71)^{f}	0.00 (0.71)^{g}	0.04 (0.74)
Haptonchus sp.	18.52 (4.34)^{a}	19.66 (4.47)^{a}	21.26 (4.65)^{a}	18.94 (4.38)^{a}	16.30 (4.06)^{a}	17.60 (4.22)^{a}	13.22 (3.66)^{a}	13.52 (3.70)^{a}	11.56 (3.40)^{a}	11.44 (3.40)^{a}	13.20 (3.65)^{a}	15.28 (3.93)^{a}	15.88 (3.99)^{c}
Nesidiocoris sp.	7.10 (2.74)^{b}	9.06 (3.07)^{b}	9.96 (3.18)^{b}	6.36 (2.56)^{b}	4.76 (2.27)^{b}	5.58 (2.41)^{b}	4.02 (2.07)^{b}	2.58 (1.75)^{b}	2.34 (1.67)^{b}	2.40 (1.68)^{b}	2.78 (1.79)	2.84 (1.79)^{b}	4.98 (2.25)^{d}
Diaphania indica	0.00 (0.71)^{e}	0.08 (0.76)^{e}	0.08 (0.76)^{g}	0.08 (0.76)^{g}	0.08 (0.76)^{e}	0.00 (0.71)e	0.04 (0.73)^{d}	0.02 (0.72)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{c}	0.06 (0.75)^{e}	0.02 (0.72)^{g}	0.04 (0.73)^{e}
Aulacophora foevicollis	0.42 (0.95)^{d}	0.36 (0.92)^{d}	0.46 (0.97)^{e}	0.40 (0.92)^{e}	0.06 (0.75)^{e}	0.06 (0.74)^{de}	0.00 (0.71)^{d}	0.02 (0.72)^{d}	0.06 (0.74)^{d}	0.06 (0.74)^{c}	0.14 (0.79)^{d}	0.16 (0.80)^{e}	0.18 (0.81)^{f}
Pachliopta hector	0.00 (0.71)^{e}	0.00 (0.71)^{f}	0.72 (1.09)^{d}	0.58 (1.02)^{d}	0.82 (1.12)^{d}	0.78 (1.11)^{c}	0.74 (1.10)^{c}	0.42 (0.93)^{c}	0.20 (0.82)^{c}	0.00 (0.71)^{c}	0.00 (0.71)^{f}	0.10 (0.76)^{f}	0.36 (0.90)^{g}
Hippotian celerio	0.00 (0.71)^{e}	0.00 (0.71)^{f}	0.00 (0.71)^{h}	0.00 (0.71)^{h}	0.00 (0.71)^{f}	0.00 (0.71)^{e}	0.00 (0.71)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{d}	0.00 (0.71)^{c}	0.00 (0.71)^{f}	1.24 (1.31)^{c}	0.10 (076)^{g}
Mean	3.34 (1.50)^{D}	3.76 (1.64)^{B}	4.20 (1.69)^{A}	3.42 (1.53)^{C}	2.88 (1.47)^{E}	3.03 (1.47)^{E}	2.26 (1.45)^{F}	2.07 (1.43)^{G}	1.77 (1.45)^{F}	1.74 (1.42)^{H}	2.09 (1.38)^{I}	2.52 (1.43)^{GH}	2.75 (1.39)
CD (5%)	Floral visitors						0.010						
	Day hours						0.012						
	Floral visitors * Day hours						0.035						

*Each value is a mean of ten observations; Figure in paranthesis are square root transformed values; In a column means followed by same letter(s) are on par with each other at 5%.

Table 4. Depicted the order abundance of different floral visitors. Among all orders Coleoptera had the highest relative abundance (72.87%), followed by Hemiptera (23.39%), Hymenoptera (2.23%) and Lepidoptera (2.29%) (Figure 2). The species evenness was relatively lower in all orders reported in this study. Even though lepidopterans had higher diversity but they were less abundantly observed. The Dominant floral visitors *Haptonchus* sp. and *Nesidiocoris* sp. are constantly associated with the bottle gourd flowers even after it closes. In bottle gourd ecosystem *Haptonchus* sp., *Nesidiocoris* sp. and *A. cerana indica* were considered as major floral visitors in Coimbatore region.

Table 4: Relative abundance of different floral visitors in bottle gourd

Order	Family	Species	Species Abundance (%)	Order Abundance (%)
Coleoptera	Nitidulidae	*Haptonchus* sp.	72.04	72.87
	Chrysomelidae	*Aulacophora foevicollis*	0.83	
Hemiptera	Miridae	*Nesidiocoris* sp.	22.61	23.39
Hymenoptera	Apidae	*Apis cerana indica*	2.03	2.23
	Halictidae	*Halictus* sp.	0.20	
Lepidoptera	Sphingidae	*Hippotion celerio*	0.47	2.29
	Crambidae	*Diaphania indica*	0.17	
	Papilionidae	*Pachliopta hector*	1.65	

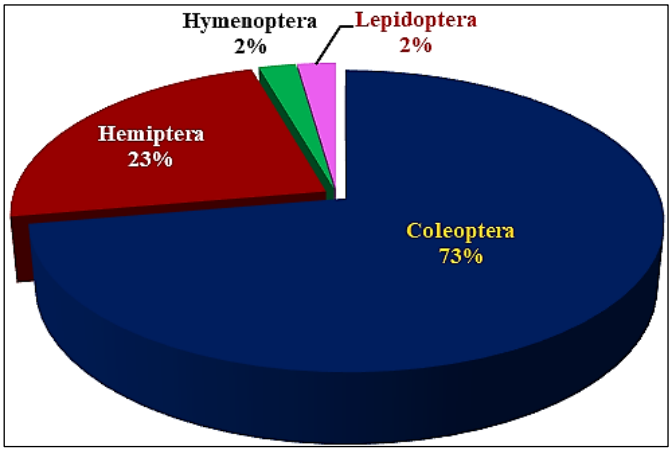

Fig 3: Relative abundance of different floral visitors

Conclusion

From the present investigation it is reported that *Haptonchus* sp was abundantly present in bottle gourd field but the pollination efficiency of coleopterans was less when compared to hymenopterans. Hence Hymenopterans and Lepidopterans were considered as major pollinators and the remaining insect species acts as minor pollinators in bottle gourd. The bottle gourd starts bloom in the late evening hours (17.00-18.00). Our suggestions is that pest management techniques should not be implemented after 1600 hours to protect pollinating insect species from the negative effects of insecticidal treatments in bottle gourd.

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