PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(1): 1-7
https://doi.org/10.14719/pst.6655

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RESEARCH ARTICLE

Unveiling the secret pollinators: A deep dive into moringas' pollination ecology

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OPEN ACCESS

ARTICLE HISTORY

Received: 11 December 2024
Accepted: 09 January 2025

Available online
Version 1.0 : 04 February 2025
Version 2.0 : 07 March 2025

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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CITE THIS ARTICLE

Saravanan P A, Ravichandran V, Veeramani P, Velmurugan M, Jayakumar J, Alagar M. Unveiling the secret pollinators: A deep dive into moringas' pollination ecology. Plant Science Today. 2025; 12(1): 1-7. https://doi.org/10.14719/pst.6655

Abstract

A famous vegetable crop in India that is rich in nutrients and heavily dependent on bees for pollination is moringa. A survey was taken in the Karur and Perambalur districts of Tamil Nadu to document the diversity of pollinators and insect visitors of moringa. The observations were analyzed using diversity indices. According to the studies, 13 hymenopteran, 7 dipteran, and 7 lepidopteran insects visited moringa plants. Fruit set and quality are maximized in moringa when pollination is successful. Therefore, four treatments have been applied to the Asiatic hive bee, *Apis cerana indica*, to explore the pollination capability and the foraging ecology of other key bee pollinators of moringa. The results affirmed that the significant *Apis* pollinators spend more time on nectar collection than pollen gathering. The peak foraging activity of *Apis* pollinators happened in the morning hours during 0900 -1100h. *A. cerana indica* and *A. dorsata* commence their foraging activity much earlier in the morning than *A. florea.* The foraging activity gradually declined after 1400h. The abundance of *A. cerana indica* was very high in managed bee pollination plots compared to open pollination plots. The managed bee pollination plots (128.2 fruits/tree) recorded 19.14 percent more fruit sets and 11.40 % more fruit yield than open pollination plots (107.6 fruits/tree) by deploying four Asian bee colonies per acre of moringa crop.

Keywords

Apis cerana indica; *Apis pollinators*; moringa; pollination

Introduction

Native to India, moringa is a versatile tropical tree. It is grown in many nations for its nutrient-rich leaves, flowers, fruits and seeds, primarily used in cooking. Recently, moringa has been considered a "superfood" that provides vital, healthy nutrients to humans to fight against many viruses (1). The trees' remarkable adaptability has earned them particular recognition. It can be used as a good source of food for both humans and animals, as a coagulant to purify water, as a treatment for various illnesses, and as a source to produce biofuel (2). In South Indian cooking, moringa pods are highly prized for their distinctively enticing flavour. Moringa blooms in terminal or axillary panicles and has fragrant, white or creamy-white flowers. *Moringa oleifera* exhibits greater fruit set, seed set, and fertility due to its adaptation to better outcrossing (xenogamy) than to selfing (geitonogamy)

(3). Moringa is cultivated on roughly 7500 hectares in Dindigul, Thoothukudi, Karur, Tiruppur, and Erode in Tamil Nadu. The moringa market is valued at over Rs.76000 crore globally and is projected to expand at 8.5 % annually to reach Rs. 145000 crore by 2030. In India, moringa trees are grown on about 43600 ha. (4)

Anthesis in moringa flowers starts in the morning, and anthers appear soon afterwards. Successful pollination by visiting bees produced an average fruit set of 10 % in moringa. Complete pollination ensures maximum fruit set and fruit quality. Each moringa flower produced an average of 23525 pollen grains, and the extended stigma receptivity promotes cross-pollination (5). Moringa produce blooms continuously twice a year, once in the summer months and later in the monsoon period. The flowers are highly cross-pollinated due to their heteromorphic nature. Moringa offers nectar and pollen as food sources for pollinators. About 90 % of pollination in most cross-pollinated plants is taken care of by honey bees (6). Pollinators are essential to 87 crops, or 70 % of the 124 significant crops grown for human consumption worldwide (7). Farmers worldwide rely heavily on insect pollination as a production method and an ecosystem function for crop development. Many species of insects were recorded as primary pollinators in moringa by many researchers, including *A. cerana indica* F., *A. florea*, *Xylocopa latipe*, *X. pubescens*, *A. dorsata* and *A. mellifera* (8, 9, 10, 11). This study aims to evaluate the diversity and foraging ecology of the primary pollinators and the impact of controlled Asian bee colonies on the fruit set and yield of moringa in Tamil Nadu.

Materials and Methods

Survey on the diversity of pollinators in the moringa ecosystem

A survey of pollinators was conducted in two districts *viz*., Pallapatti of Karur district (10.7234 °N; 77.9043 °E) and Veppanthattai of Perambalur district (11.3511 °N; 78.8039 °E) during 2020-22. Each plot has five randomly chosen moringa trees designated. Five inflorescences from each randomly chosen tree were chosen to examine pollinators' diversity. In each of the five trees, pollinator visitation in five inflorescences was observed for an average of every five min. The final data was reported as pollinator count/25 inflorescences/five min. The data was collected hourly between 6 am and 6 pm.

Shannons' diversity index (H)

The pollinators' diversity was estimated using Shannons' index (12). Shannons' index accounts for both the abundance and evenness of the species present. Shannons' diversity index was calculated using the following formula in Equation 1.

$$H = \sum_{i=0}^{S} pi \ln pi \quad \text{.....(Eqn. 1)}$$

Species richness (S), the total number of species in a community, was assessed. Shannons' equality (E_H) was calculated by dividing H by H_{max} (here H_{max} = lnS). Equality assumes a value between 0 and 1, being complete evenness and was calculated using the following formula in Equation 2.

$$EH = \frac{H}{H\,max} = \frac{H}{\ln(S)} \quad \text{.....(Eqn. 2)}$$

Simpsons' diversity index

Simpsons' diversity index characterizes species diversity in a community. The proportion of species *i* relative to the number of species (*pi*) was calculated and squared. It was calculated using the following formula in Equation 3.

$$D = \frac{1}{\sum_{i=1}^{S} pi2} \quad \text{.....(Eqn. 3)}$$

Equitability (ED) was calculated by taking Simpsons' index (D) and it as a proportion of maximum value D could assume if individuals in the community were evenly distributed (D_{max}), which equals S and was calculated using the following formula in Equation 4.

$$ED = \frac{D}{D\,max} \quad \text{.....(Eqn. 4)}$$

Foraging ecology of major bee pollinators in moringa

Ten plants were chosen randomly, three inflorescences were tagged, and observations were made on each plant to record the foraging task of pollinators.

Initiation and cessation time of foraging

The beginning and ending of pollinators' foraging activities were timed on specific flowers. The pollinators' overall working period was computed over ten days per day during the peak flowering period and the mean was determined.

Flower handling time

Five observations were made on randomly chosen days throughout the peak flowering period, and the time spent by individual bee pollinators/flower/min on an inflorescence for the collection of rewards, namely pollen and nectar, was recorded using a stopwatch.

Peak foraging activity

The total number of foragers visited on moringa flowers was recorded. The flowers in selected three inflorescences were observed hourly from 6:00 am to 6:00 pm for 5 min. The number of foragers was counted at fortnightly intervals during the two to three months of flowering.

Abundance of major bee pollinators

An abundance of major bee pollinators was recorded as no. of foragers visited/inflorescence/ 5 min in both managed and control plots during the peak foraging activity period.

Managed bee pollination experiment

The field tests were conducted using a randomized block design with four treatments, including a control with five replications, to investigate the pollination function of

A. cerana indica. The experiment was conducted in a few farmers' fields in Veppanthattai and the adjacent areas during February 2021. In Veppanthattai village, Perambalur District, four bee colonies (T_1) were kept in a one-acre moringa field. Two more colonies (T_2) were established in a farmers' field in Venbavur village, and a control plot (open pollination T_3) was maintained in Veppanthattai village. Using nylon net sleeve cages, pollinators were kept out of randomly chosen inflorescences (bud stage) from a moringa plot (T_4) selected from Veppanthattai village. The number of fruits produced per tree, flower visitors, bee visitation rate, fruit length (cm), fruit girth (cm), fruit weight (g), and total fruit weight per tree (kg) were all recorded regularly.

Results

Pollinator diversity in moringa

The observations presented in Table 1 showed that 27 pollinator and insect visitor species have been identified and documented in the moringa crop environment. Of them, seven were Dipteran, seven were Lepidopteran, and thirteen were Hymenopteran. The Non-Apis group of hymenopterans includes ten species from the Vespidae, Scoliidae, Sphecidae, and Pompilidae. In comparison, the Apis group comprises three species: *Apis cerana indica, Apis dorsata,* and *A. florea.* The Syrphidae, Sarcophigidae, Calliphoridae, Dolichopodidae, and Muscidae are the seven insect families that comprise the Dipterans. Papilionidae, Pieridae, Nymphalidae, Hesperidae, and Sphingidae are the families of seven insect species among lepidopterans.

The recorded pollinators and insect visitors were categorized into *Apis* sp., Non-*Apis* Hymenopterans, Dipterans and Lepidopterans (Table 2). With a mean of 4.95/25 inflorescence/5 min, *Apis* sp. were the most numerous, followed by Non-Apis Hymenopterans (3.37), Dipterans (1.56), and Lepidopterans (0.56). *Apis cerana indica* was the primary pollinator among Apis Hymenopterans, with a mean of 3.36/inflorescence/ 5 min, followed by *Apis dorsata* and *Apis florea*. *Amegilla cingulata* (1.51) was the primary pollinator of non-Apis Hymenopterans, followed by *Xylocopa* sp., *Polistes* sp., and *Scolia* sp. The most common Dipteran visitor was *Episyrpus*, followed by *Sarcophaga* sp. and *Lucilia papuensis*. The skipper (0.13/ inflorescence/5min) was the most frequent insect visitor among the Lepidopterans, followed by *Danaus chrysippus*

Table 1. Pollinator and insect visitor diversity in moringa.

S. No	Pollinators	Systematic position	Role (N / P / N+P)
1	*Apis cerana indica*	Hymenoptera: Apidae	N+P
2	*A. dorsata*		N+P
3	*A. florea*		N+P
4	*Amegilla zonata*		N+P
5	*Amegilla quadrifasciata*		N+P
6	*Xylocopa* sp.		N+P
7	*Polistes* sp.	Hymenoptera: Vespidae	N
8	*Vespa orientalis*		N
9	*Scolia* sp.	Hymenoptera: Scoliidae	N
10	*Sphex* sp.	Hymenoptera: Sphecidae	N
11	*Megachile* sp.	Hymenoptera: Megachilidae	N+P
12	*Pepsis* sp.	Hymenoptera: Pompilidae	N
13	*Halictus* sp.	Hymenoptera: Halictidae	N+P
14	*Episyrpus* sp.	Diptera: Syrphidae	N
15	*Sarcophaga* sp.	Diptera: Sarcophigidae	N
16	*Lucilia papuensis*	Diptera: Calliphoridae	N
17	*Eristalinus arvorum*	Diptera: Syrphidae	N
18	*Condylostyus* sp.	Diptera: Dolichopodidae	N
19	*C. occidentalis*	Diptera: Dolichopodidae	N
20	*Musca domestica*	Diptera: Muscidae	N
21	*Papilio polytes*	Lepidoptera: Papilionidae	N
22	*Pieris rapae*	Lepidoptera: Pieridae	N
23	*Tirumala limniace*	Lepidoptera: Nymphalidae	N
24	*Hypolimnas bolina*		N
25	*Danaus chrysippus*		N
26	*Oriens goloides*	Lepidoptera:Hesperidae	N
27	*Sphinx* sp.	Lepidoptera: Sphingidae	N

N-Nectar; **P**-Pollen.

Table 2. Pollinator population in moringa on different days of blossom.

Pollinator groups / Days*	Pollinator population in 25 inflorescences / 5 min*									
	Day1	Day2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Apis Hymenopterans	3.73	6.18	7.09	6.91	3.64	4.09	3.73	4.18	4.09	4.09
Non-*Apis* Hymenopterans	2.36	2.45	3.55	3.27	5.09	3.73	3.45	3.36	3.73	3.45
Dipterans	1.45	1.36	1.64	1.27	0.91	1.55	1.91	1.55	1.64	1.82
Lepidopterans	0.27	0.09	0.36	0.36	0.91	0.91	0.82	0.55	0.73	1.00
Diversity indices										
Species richness (S)	4	4	4	4	4	4	4	4	4	4
Simpsons' D	2.82	2.21	2.43	2.32	2.73	3.12	3.26	2.95	3.07	3.26
Simpsons' E	0.70	0.55	0.61	0.58	0.68	0.78	0.81	0.74	0.77	0.81
Shannons' H	1.14	0.96	1.05	1.02	1.14	1.23	1.26	1.19	1.22	1.26
Shannons' E	0.82	0.69	0.76	0.73	0.82	0.89	0.91	0.86	0.88	0.91

and *Papilio polistes*. Pollinator visits to inflorescences for five min, which acted as replications, were recorded each hour from six in the morning to six in the evening for 10 days at peak flowering. The Simpsons' D diversity index ranged from 3.3 to 7.6 throughout the day, with the highest value (7.6) occurring between 2 and 3 pm. There was increased species richness (S) between 9 and 10 am and 1-2 pm.

Foraging ecology of major hymenopteran pollinators

The foraging activity of *A. cerana indica* and *A. dorsata* commences much earlier at 0520 to 0540 h and *A. florea* starts late by 0630 h. *A. florea* foraging was halted by evening 1740 h compared to *A. dorsata* and *A. cerana indica* which ceased their foraging activity by 1810 and 1815 respectively (Table 3). Generally, all three Apis bees spent more time gathering nectar than pollen foraging. The workers of *A. cerana indica* and *A. dorsata* comparatively spent a shorter time for nectar collection, 14.36 sec/ flower and 12.26 sec/flower, respectively, in an inflorescence than A. *florea,* which took a much longer period of 23.46 sec/flower. Similarly, *A. florea* spent a comparatively longer duration (9.4 sec/flower) than *A. cerana indica* and *A. dorsata* for pollen collection (Table 3).

Table 3. Initiation, cessation and flower handling time of major bee pollinators in moringa.

Species	Flower handling time (sec)*		Foraging time	
	Nectar	Pollen	Initiation	Cessation
Apis cerana indica	14.36±1.05	6.2±1.17	0540 h	1815 h
Apis dorsata	12.26±1.05	3.4±0.60	0520 h	1810 h
Apis florea	23.46±2.2	9.4±1.40	0630 h	1740 h

Peak foraging activity of *A.cerana indica*

The findings of the peak foraging activity of major bee pollinators of moringa were presented in Fig.1. The results indicated that the peak foraging activity of *A. cerana indica* (number of foragers/ inflorescence/min) was noticed during 0900-1000 h (4.50) and 1000-1100 h (4.60). The foraging activity gradually declined after 1300-1400 h (0.20) and no activity was observed between 1400-1500 h. Similarly, the peak foraging activity of *A. dorsata* was observed between 0800 -0900h (2.6) and no activity was recorded during 1300 -1400h. Peak foraging in *A.florea* was distinct and noticed between 1000-1100h (6.26) and it gradually declined in the afternoon between 1500-1600h (0.34).

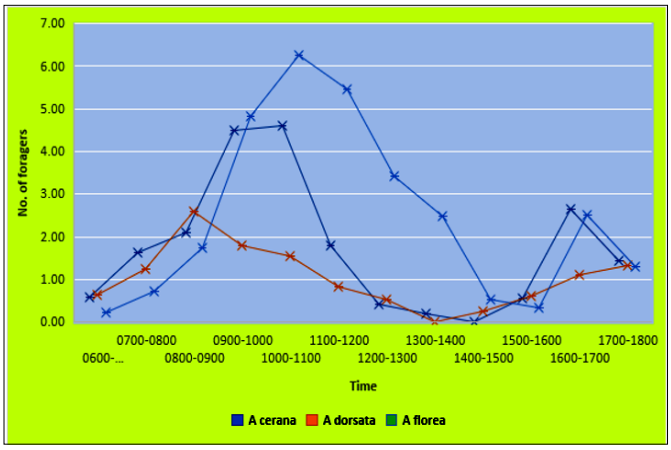

Fig. 1. Peak foraging activity of major pollinators in moringa.

Abundance of *A. cerana indica* in moringa flowers

The observations on bee abundance revealed that *A. cerana indica* mean visitation rate was more prominent in the managed bee plot, with 14.60 bees/inflorescence/ 5min compared to the control plot (4.8 bees/ inflorescence/ 5 min, indicating the potential of keeping the managed Asiatic hive bee colonies. The *A. dorsata* bee activity was moderately higher in the control plot (3.2 bees) than in the managed bee plot (2.8 bees). Similarly, *A. florea activity* was maximum in the control plot (6.4 bees/ inflorescence/ 5 min) than in the managed bee plot with 5.2

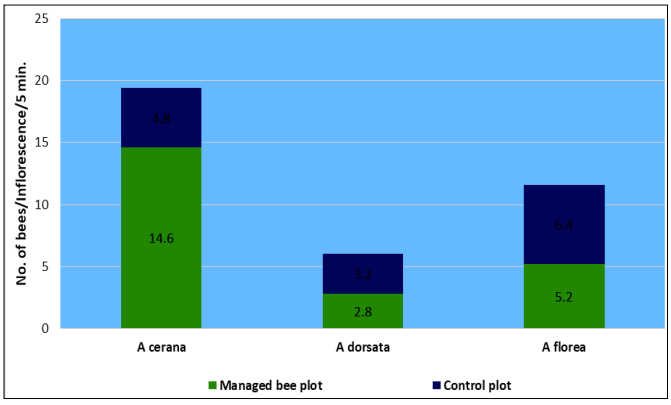

Fig. 2. Abundance of major pollinators in moringa.

bees/ inflorescence/ 5 min. (Fig 2).

Managed bee pollination in moringa

The findings of the field studies, which were carried out at various sites, showed that the *A. cerana indica* bee visitation rate/inflorescences /3 min was highest (6.02) in T_1 than T_2 (4.00) and T_3 (2.2). The T_1 and T_2 plot also registered significantly better fruit length (54.30 cm, 49.74 cm), fruit girth (6.38 cm, 6.12 cm) and fruit weight (126.4 g, 119.8 g), respectively, compared to the T_3 plot (Table 4). Similarly, compared to the T_3 (107.6), the number of fruit set per tree was significantly higher in T_1 (128.2) and T_2 (122.8). The introduction of four *A. cerana indica* bee colonies per acre increased considerably fruit yield and fruit set compared to the control plot by 11.40 and 19.14 percent, respectively.

Table 4. Impact of *A. cerana indica* pollination on moringa yield and yield factors.

	Fruit length (cm)	Fruit girth (cm)	Fruit weight (g)	No. fruits / tree	% increase over control	Total fruit weight (Kg/tree)	Bee visitation rate (No./inflorescence/3min.)
T_1 – 4 bee colonies /acre	*54.30^a	6.38^a	126.4^a	128.2^a	19.14	16.20	6.02^a
T_2 – 2 bee colonies /acre	49.74^{ab}	6.12^{ab}	119.8^a	122.8^b	14.13	14.71	4.00^b
T_3 – Control (no managed bee colonies)	43.12^b	5.94^b	109.2^b	107.6^c	0	11.75	2.20^c
T_4 – Pollinator exclusion#	0	0	0	0	-	0	0
SE (d)	2.97	0.29	5.36	6.43	-	-	0.29
CD (0.05)	6.48	0.63	11.69	14.0	-	-	0.64

*Mean of five replications; means followed by different letters within a column indicate significant differences (P<0.05); #Observations only from the bagged inflorescences.

Discussion

Moringa bloom is very attractive for more insect visitors and pollinators. A total of 27 insect species, mainly from Hymenoptera, Diptera and Lepidoptera, were recorded on moringa flowers (Fig. 3). *A.cerana indica* was found as the dominant visitor, followed by other apis and non-apis bees. The high density of natural colonies and adaptation of A.cerana indica in these areas could make them dominant native pollinators. Additionally, (13) noted that *Scolia dubia* S. (30.22 %), *Xylocopa virginica* L. (21.24 %) and other insects were the most frequent visitors to the drumstick flower. Eight insect species were observed visiting the blooms of *M. concanensis nimmo* Linn (14). The primary pollinators recorded were the black ant (*Camponotus compressus* F.) and the small bee (*A. florea*). Moringa blooms attract a more significant number of floral visitors. Pollinators of moringa flowers include honey bees, carpenter bees, and other wild bees (15). Generally, the *Apis* species has better morphological features like pollen basket-dense body hair than other bee species. They have a unique edge over other bee species, operating in diverse environments with temperature tolerance, longer foraging range, and high floral fidelity (16).

The flower handling time of primary pollinators depends on the type of floral rewards they collect from moringa flowers. Nectar foragers spend more time than pollen foragers. The moderately smaller population of wild bees noticed in managed bee plots may be due to inter-species competition from managed *A. cerana indica* colonies. Primary bee pollinators' relative abundance and peak foraging activity were higher during the morning than in the afternoon (Fig. 2). These results indicate a remarkable variation in the distribution of peak foraging activities between the three major bee pollinators in moringa. The mean number of insect pollinators visiting the moringa bloom was highest between 0900-1200h (11). Similarly, research indicates that the pollen collection from moringa flowers by *A. florea* was more at about 1000 h. and less at about 1600 h (14). The carpenter bees were found to be visiting drumstick flowers most often in the early morning hours and the afternoon between 4 to 6 pm (14).

Honeybees play a crucial role in pollination and fruit sets of moringa crops. Compared to open pollination and control plots, deploying four *A. cerana indica* bee colonies per acre of moringa enhanced fruit set and yield. This may be due to the frequency of *A. cerana indica* bee visitation (6.02) in managed bee plots than in open pollination plots (2.20). Similarly, in bitter gourd pollination, the number of fruits/plant (17.4 fruits) and fruit yield per hectare (41.13 T/ha) was higher in *A.cerana indica* bee pollinated plot than in open pollination plots (16.2 fruits) and (37.25 T/ha) reported in Tamil Nadu by (17). Research also highlighted the importance of carpenter and honey bees in moringa production (3). Research indicates the various moringa pollinators and found that honey bees were highly efficient (18). The highest number of seeds set (yield) after bee fertilization of moringa had been achieved (19).

Conclusion

Apis cerana indica are highly efficient pollinators of moringa flowers. These bees are highly attracted to the nectar and pollen produced by the moringa flowers. The bees fly from flower to flower to collect rewards and transfer pollen, facilitating the fertilization and fruit set. The high abundance, efficient floral handling, and fidelity characters underscore them as ideal pollinators in the moringa ecosystem. Establishing four Asian bee colonies per acre of moringa crop enhanced the fruit set (19.14 %) and yield (11.40 %) compared to open pollination and control plots. *A. cerana indica* is a beneficial pollinator and valuable input for growing moringa. Through comprehension of its

Fig. 3. Diversity of pollinators in moringa. (**A**)*Apis dorsata* ; (**B**)*Apis cerana indica* ; (**C**) *Apis florea* ; (**D**) *Xylocopa* sp.; (**E**) Amegilla zonata and (**F**) *Papilio polytes* .

function in pollination and its effect on yield, farmers and researchers may put plans into place to enhance moringa production and yield by optimizing the pollination services of *A. cerana indica.* Protecting and promoting the populations of these native bees is essential for ensuring the long-term viability of moringa farming and its associated benefits. Hence, placing four *A. cerana indica* bee colonies in an acre of moringa crop during the blooming periods could

enhance the moringa fruit set by 19.14 % and yield by 11.40 %.

Acknowledgements

The authors are grateful to ICAR for the financial support rendered through the AICRP Honey Bee and Pollinators project. The authors also wish to acknowledge the immense help received from the scholars whose articles are cited and included in the references of this manuscript.

Authors' contributions

PAS carried out the field experiments and all observations and drafted the manuscript. VR contributed to language editing and manuscript correction. PV contributed to the design of the study and performed the statistical analysis. MV participated in the documentation of various pollinators. MA participated in identifying insect visitors and coordinating the study, and JJ contributed to manuscript correction.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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