Original Research Article

Relative contribution of bee pollinators in Aster (*Callistephus chinensis* L.) pollination

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ABSTRACT

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| **Aims:** Many insects visit flowering plants, but it’s important to distinguish between floral visitors and effective pollinators, which is often overlooked. There is no such information available about the pollinators of Aster (*Callistephus chinensis*), a popular seed-propagated flowering crop in India. This study aims to assess the contribution of bee pollinators to the plant’s reproductive success.  **Place and Duration of Study:** The study was conducted over two years (2021-22 and 2022-23) at the ICAR-Directorate of Floricultural Research, Pune.  **Methodology:** Observations were recorded at the 50 per cent flowering stage during peak pollinator activity (11:00-12:00 hrs). To assess the relative significance of frequent floral visitors as pollinators, the pollination importance index (PII) was calculated using four factors: (1) Relative abundance (A), (2) Pollen-carrying capacity (PCC), (3) Host fidelity (F) and (4) Pollinator effectiveness (PE).  **Results:** The results showed that *Apis florea* was the predominant pollinator of Aster (57.58%) followed by *Apis cerana indica* (23.93%). While, the highest pollen-carrying capacity was observed in *A. cerana indica* (4.68 mg per bee) followed by *A. florea* (3.88 mg/bee). All species showed complete host fidelity to the aster and observed to contact both the stigma and the anther while foraging on aster. The highest Pollination Importance Value (PIV) and Pollination Importance Index (PII) was recorded for *A. florea* (223.31, 64.77, respectively), followed by *A. cerana indica* (111.92, 32.44, respectively). While other species show comparatively lesser values.  **Conclusion:** The PIV and PII indicated that *A. florea* was the most efficient pollinator of Aster compared to *A. cerana indica.* Other pollinators also contributed to Aster pollination but in lesser extent. Therefore it is also necessary to conserve other pollinators along with honey bees. These findings provide valuable insights for optimizing pollination management in commercial Aster cultivation, promoting the use of effective pollinator species to improve crop yields. |

*Keywords: Aster,**pollinator importance index, pollination importance value, pollen-carrying capacity, relative abundance*

1. INTRODUCTION

A wide variety of insects visit flowering plants (Sihag, 1986); some are nectar robbers, some act as pollinators and some play both roles (Sihag, 1988; Sihag, 2018). Their abundance differs from high to low and their foraging speeds vary from very fast to extremely slow (Sihag, 2018; Saini and Sihag, 2023). By considering these factors, not every insect that visits flowers is as important to the plant as the others (Sihag, 2018). This is because each of these visitors contributes differently to a plant's reproductive success Bottom of Form

(Sihag, 2018). Some visitors are highly efficient at transferring pollen from the anthers to the stigma, while others are either inefficient or less efficient. This pollen transfer efficiency is expressed as visitor's pollination efficiency which represents their contribution to the plant's reproductive success (Saini and Sihag, 2023). It can be evaluated by direct or indirect methods (Adlerz, 1966; Beattie, 1971).

In certain plant species like Asteraceae it is difficult to directly assess pollinator effectiveness due to its flower structure (Lindsey, 1984), for example, it is difficult to identify which stigmas are ready to receive pollen and which ones have already received xenogamous pollen. In these situations, looking at the overall effectiveness of the floral visitors as pollinators can offer valuable insights into the plant’s pollination ecology.

Pollinators effectiveness influenced by a variety of factors. These factors include the pollinator's morphological traits, which allow for pollen collection and transfer, its foraging behaviour, and its degree of fidelity to a particular plant species. In addition to that, the relative abundance of a pollinator compared to other visitors, as well as the amount of time it spends foraging on a plant's flowers, contribute to its overall pollination efficiency (Silander and Primack, 1978; Bertin and Willson, 1980; Gyan and Woodell, 1987; Herrera, 1987). The floral morphology of the plant i.e. accessibility of their reproductive organs are also plays a key role in determining the success of pollination (Bohart and Nye, 1960; Beattie, 1971). By combining factors such as foraging behaviour, pollen-carrying capacity, visitor abundance and other relevant traits, ‘pollinator index’ or importance value can be determined. This index helps rank different pollinator species based on their effectiveness in pollination, which offers a way to assess their contribution to plant reproduction (Ehrenfeld, 1979; Lindsey, 1984).

There is no such information available about the pollinators of Aster, *Callistephus chinensis* which is a popular seed-propagated flowering crop in India (Chakraborty *et al.*, 2019). It is commercially cultivated for its cut and loose flowers which are used for garlands, bouquets and flower decoration. It is the third most popular flower in India due to its multiple uses and ease of growing (Bhargav *et al*., 2016). The popularity of Aster flowers increasing day by day as a result the demand for high-quality seeds also increases.

*Callistephus chinensis* is ageitonogamous crop i.e. it required pollinating agents to transfer pollen grains from the anther of one flower to the stigma of another flower of the same plant (Sheela, 2008). In Aster, single and semi-double varieties are primarily cross pollinated (Strube, 1965; Janakiram, 1997) that they rely primarily on pollinators for effective seed production. Aster flowers are visited by many different insect species because of their bright colours (Horsburgh and Semple, 2011). Many studies have demonstrated that proper pollinator management can significantly maximize seed yields in a wide range of crops (Melnichenko and Khalifman, 1960).

Therefore, the study aims to assess the relative significance of bee pollinators in aster, so it will help in improving the pollination management in aster by promoting the use of effective pollinator species to increase the aster seed yields.

2. material and methods

An experiment was conducted during 2021-22 and 2022-23 at the ICAR-Directorate of Floricultural Research, Pune in the widely cultivated aster variety (*cv*. Phule Ganesh Pink). Crop was grown following all recommended agronomic practices, except plant protection measures. The experiment was carried out in a 5 m x 5 m plot on raised beds, with a plant spacing of 45cm x 60cm. Observations were recorded at the 50 per cent flowering stage, during the peak pollinator activity period from 11:00 hrs to 12:00 hrs. The bee pollinators which were frequently observed visiting the flowers were observed to assess their relative significance in aster pollination.

**2.1 Observations recorded**

To evaluate the relative significance of bee pollinators, the pollination importance index (PII) for each pollinator was determined, based on methods from Lindsey (1984) and Mochizuki and Kawakita (2018). The PII is calculated using four main factors: (1) Relative abundance (A), (2) Pollen-carrying capacity (PCC), determined by the weight of pollen carried, (3) Host fidelity (F), which determined by the average percentage of pollen that comes from the host plant and (4) Pollinator effectiveness (PE), which estimates the probability that a specific visitor's foraging behaviour will result in successful pollination (Lindsey, 1984; Mochizuki and Kawakita, 2018).

**2.1.1 Relative abundance**

Observations were recorded by counting the total number of each species visiting flowers within a 1m×1m area in five minutes from 11:00 hrs to 12:00 hrs. A stopwatch and a hand tally counter were used to determine timing and counting (Free, 1993).

Relative abundance (%) was calculated using the formula,

Relative abundance (%) = Ni/N × 100

Where, Ni represents the number of individuals of a single species and N represents the total number of flower visitors visiting the crop.

**2.1.2 Pollen-carrying capacity (PCC)**

The pollen-carrying capacity (PCC) of bee pollinators was assessed by measuring the weight of the pollen they carried. Twenty specimens of each species were trapped randomly in plastic vials, anesthetized with ethyl acetate vapours and their pollen loaded body weighed using an electronic balance (Model-ATX224, Shimadzu, Kyoto, Japan). After brushing the pollen off, the weight was measured again and the difference indicated the amount of pollen carried (Akhter *et al*., 2016).

**2.1.3 Host fidelity (F)**

This is represented by the average percentage of the pollen load that contains host plant pollen. Host plant pollen grains were identified by comparing them with a pollen reference from plants that were flowering at the study site.

**2.1.4 Pollinator effectiveness (PE)**

We assigned a PE value of 1 to pollinators contacting both stigma and anther, and a value of 0 to those contacting neither or only one of the parts (Mochizuki and Kawakita, 2018).

**2.1.5 Pollination importance value (PIV)**

Similar to the methods used by Lindsey (1984) and Mochizuki and Kawakita (2018), pollination importance value (PIV) was determined for each bee pollinator group by multiplying four main values,

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PIV = A × PCC × F × PE.

Where, A = Relative abundance; PCC = Pollen-carrying capacity, F = Host fidelity and PE = Pollinator effectiveness.

**2.1.6 Pollination Importance Index (PII)**

To evaluate the relative pollination importance among the major bee polllinators, we assigned a PII score to each functional group using the following calculation,

PII = (PIV/ƩPIV) × 100 (Lindsey, 1984)

Where,

PIV = Pollination importance value of each species;

ƩPIV = Sum of pollination importance value of all species

**2.2 Statistical analysis**

All the data regarding relative abundance, pollen-carrying capacity, host fidelity, pollinator effectiveness, pollination importance value and pollination importance index were statistically analyzed using analysis of variance for a randomized block design after appropriate transformations where necessary (Gomez and Gomez, 1984).

3. results

The data recorded during 2021-22 and 2022-23 indicated that *A. florea* was the predominant pollinator of Aster, contributing an average of 57.58% of bee population/ m²/ 5 min, followed by *A. cerana indica* at 23.93%, *Ceratina hieroglyphica* at 5.79% and *Ceratina binghami* at 4.95%. *Lassioglossum leaucozonium* (4.09%) and *Braunsapsis* sp. (3.66%) were observed visiting the flowers less frequently, with no statistically significant difference among their abundance (Table 1).

The highest pollen-carrying capacity was observed in *A. cerana indica*, with an average load of 4.68 mg per bee followed by *A. florea* (3.88 mg/bee) and *L. leaucozonium* (0.79 mg/bee). *C. binghami* (0.47 mg/bee) and *C. hieroglyphica* (0.46 mg/bee) showed comparable pollen carrying capacity, with no statistically significant difference between them. *Braunsapsis* sp*.* carried the least amount of pollen, with an average of 0.37 mg/ bee (Table 1).

In terms of host fidelity, the average proportion of pollen load consisting of host pollen was 1 for all major bee pollinator species observed on Aster, including *A. florea*, *A. cerana indica*, *L. leaucozonium*, *C. hieroglyphica*, *C. binghami* and *Braunsapsis* sp. This indicates complete host fidelity (F), as the pollen loads of these species were entirely composed of pollen from the aster flower (Table 1).Top of Form

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The Pollinator Effectiveness (PE) value for all major bee pollinators was assigned a value of 1, as each species was observed to contact both the stigma and the anther while foraging on aster which indicates full effectiveness in pollination for all species (Table 1).

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| **Species**  **Table 1: Pollination Importance Value of major bee pollinators in aster (Pooled data of 2021-22 and 2022-23)** | **Relative abundance**  **(A)** | **Weight of pollen (mg) (PW)** | **Pollination importance value (PIV)** | **Pollination importance index (PII)** |
| ***Apis florea*** | 57.58  (49.36)a\* | 3.88b | 223.31a | 64.77  (53.60)a\* |
| ***Apis cerana indica*** | 23.93  (29.27)b | 4.68a | 111.92b | 32.44  (34.71)b |
| ***Lassioglossum* *leaucozonium*** | 4.09  (11.55)e | 0.79c | 3.25c | 0.95  (5.51)c |
| ***Ceratina hieroglyphica*** | 5.79  (13.87)c | 0.46d | 2.64c | 0.77  (5.02)c |
| ***Ceratina binghami*** | 4.95  (12.83)d | 0.47d | 2.30c | 0.67  (4.69)d |
| ***Braunsapsis* sp.** | 3.66  (10.98)e | 0.37e | 1.36c | 0.40  (3.59)e |
| **S.E(m)±** | 0.29 | 0.02 | 1.59 | 0.25 |
| **CD at 5%** | 0.81 | 0.06 | 5.02 | 0.71 |

\*Figures in parenthesis are Arc sine transformed values

\*In a column, means followed by same alphabet do not differ significantly (p= 0.05) by DMRT

The highest Pollination Importance Value (PIV) was recorded for *A. florea* (223.31), followed by *A. cerana indica* (111.92). *L. leaucozonium* (3.25) which was statistically similarto *C. hieroglyphica* (2.64), *C. binghami* (2.30) and *Braunsapsis* sp. (1.36) showed lower PIVs (Table 1).

The highest Pollination Importance Index (PII) was recorded for *A. florea* (64.77), followed by *A. cerana indica* (32.44). Other species in order was *L. leaucozonium* (0.95) which was comparable to *C. hieroglyphica* (0.77) and *C. binghami* (0.67), all showing progressively lower indices with no statistically significant difference among them. The lowest PII was observed in *Braunsapsis* sp. (0.40) (Table 1).

4. discussion

The variations in Pollination Importance Value (PIV) among different pollinators can be due to several factors, including their abundance, their capacity to carry pollen, their fidelity to particular plant species, and their overall pollination efficiency (Silander and Primack, 1978; Bertin and Willson, 1980; Gyan and Woodell, 1987; Herrera, 1987). These factors are also used to calculate the Pollination Importance Index (PII), which ranks pollinators by their effectiveness in plant reproduction (Ehrenfeld, 1979; Lindsey, 1984).

However, there remains a research gap regarding the contribution of different insect visitors in aster pollination. So, in this study, similar researches from other crops were used to discuss and compare our findings.

The predominance of *A. florea* followed by *A. cerana indica* was also observed by Gupta *et al.* (2020) in asteraceae plants. However, in contrast Sajjanar *et al*. (2023) and Padhy *et al.* (2024) reported dominance of *A. cerana* over *A. florea* in sunflower crop. Our result was also in conformity with Goswami *et al*. (2013) and Rasheed *et al*. (2015) who reported that *Apis* species were more dominant than non-*Apis* bees in sunflower, with Halictidae bees making a minimal contribution in pollination.

Honeybees are the most common visitors in aster fields because of their large colonies that allow them to visit flowers frequently (Seeley, 2009). Unlike other bees, honeybees forage across multiple flowers to gather food to support their colony (Müller *et al*., 2006). Their flower constancy, efficient communication (e.g., waggle dance) and optimal size make them more abundant and effective pollinators (Free, 1963; Robinson and Morse, 1989; Chittka *et al*., 1999; Slaa *et al*., 2003).

The pollen carrying capacity of bees varied with respect to species of bee and the plant they foraged on (Lukoschus, 1957). The higher pollen carrying capacity in *A. cerana indica* compared *A. florea* and other non-*Apis* bees may be due to their larger body size than other bees as there is a positive correlation between bee size and pollen load (Kitroo and Abrol, 1996). Our result was also in conformity with the Yadav *et al*. (2024) reported that *A. cerana* carried the maximum number of loose pollen grains as compared to *A. florea* in apple ber. The result was also align with the Padhy *et al*. (2021), reported that *A. cerana indica* carried the maximum pollen loads in winter, with 5 mg in the first year and 4.8 mg in the second year. The higher pollen carrying capacity in honeybees than other pollinators is due to their special morphological adaptations like dense body hairs and corbicula (pollen baskets) on their hind legs which makes easy for them to collect and carry larger quantities of pollen on their body (Thorp, 1979).

Complete host fidelity in bees for Aster showed that aster is a rich source of nectar and pollen. Some studies also showed that under most rewarding conditions honeybees show greater flower constancy with nearly 100 per cent (Grüter *et al*., 2011). Pure pollen in bees may also be due to predominance of aster in field (Brittain and Newton, 1933). Frankie *et al*. (2005) found that asteraceae was the most commonly visited plant family by 76 species of native bees. Honeybees possess flower constancy due to their memory and learning abilities (Hill *et al*., 1997). This behaviour tends to visit only one flower species per foraging trip, leading to pure pollen loads in bees. Bees usually stick to one flower type because it's easier for them to remember and switching flowers takes extra effort to learn the new flower, which reduces its energy efficiency (Chittka *et al*., 1999).

Honeybees use the waggle dance to communicate food location and quality with nest mates, which helps to maintain constancy (Chittka *et al*., 1999). This reduces the chances of foragers switching to other sources (von Frisch, 1967; Seeley, 1983; Seeley, 1995; Biesmeijer and Seeley, 2005; Riley *et al*., 2005; Grüter and Farina, 2009; Grüter *et al*., 2010). As a result, honeybees show high constancy, especially when rewards are abundant (Grüter *et al*., 2011).

The flower development pattern in aster allows bees to contact both the stigma and anther. As the anther filaments elongate, pollen grains are released inside the anther tube. Pollen is released and moves to other parts of the flower for pollination. During the flower transition stage, the stigma extends through the anther lobe. Aster plants have secondary pollen presentation, where pollen moves from the anther to other parts of the flower, making it ready for pollination. The pollen left in the anther tube sticks to the hairs on the stigma, and this pollen is then pushed out and exposed, making it accessible to pollinators (Hong *et al*., 2008). During the pistillate stage, the stigma grows in two steps: first, it elongates, then it separates in the middle and curls the tip outward (Sammataro *et al*., 1985). This allows bees to touch both the exposed pollen and stigma while foraging, which helps in aster pollination (Sharma *et al*., 2014).

*Apis* bees forage for nectar, pollen or both from aster flowers. When collecting nectar, the bee’s body becomes nearly vertical and its tongue and head insert into the corolla tube. Afterward, the bee cleans off pollen from body with its legs, transferring it to the flower’s stigma and helping in pollination. Before leaving, bee cleans off pollen from its body with its legs. In this process, pollen grains sticking to the facial region of the bee and come into close contact with the stigmatic surface of the flower. This interaction directly helps in pollination. In addition to nectar, bees also collect pollen by walking over the head of the flower using their forelegs and middle legs to gather pollen. Pollens often accumulate on the bee’s body and in some foragers, especially on the dorsal side of the thorax (Ramya *et al.* 2014). Simialar foraging behaviour in case of *Apis* bees and *Ceratina* sp. was also observed by Ramya *et al.* (2014) and Moussa *et al*. (2022) in sunflower.

Several researches showed that *Apis* species especially *A. florea* and *A. cerana indica* has significant contribution in pollination of various crop due to their high abundance, significant pollen carrying capacity, flower constancy and their pollination efficiency. While other species do contribute to pollination, but their roles are relatively minor.

Kachhawa *et al*. (2020) reported that *A. florea* was more abundant than *A. cerana* on *Tagetes erecta* flowers. They also found that although *A. cerana* carried more pollen grains than *A. florea*, *A. flore*a had a significantly higher pollination index (84,325.50) compared to *A. cerana* (48,850.52). This suggests that, despite carrying fewer pollen grains, *A. florea* was more efficient at pollinating because it was more abundant than *A.cerana*. Similar kind of observation was also recorded by Kitroo and Abrol (1996) and Yadav *et al.* (2024) inlitchiand apple ber, respectively.

The importance of higher abundance in the plant's reproductive success was also reported by Olsen (1997) in *Heterotheca subaxillaris*. Similarly, based on the abundance of insect pollinators and the pollen carrying capacity, Strange *et al*. (2020) identified  *Halictus ligatus* (sweat bees), and *Lasioglossum* /*Dialictus* sp. as primary pollinators of *Helianthus verticillatus*.

Horsburgh and Semple, (2011) also reported that honeybees had the highest potential pollinator effectiveness (PPE) on *Symphyotrichum lanceolatum* due to their high abundance, but their low abundance on *S. lateriflorum* reduced their effectiveness. Halictid bees, although carrying similar pollen loads on both species, spent more time foraging on *S. lateriflorum* (PPE 220.1 vs. 121.1). Longer foraging times lead to better pollen deposition, which makes Halictidae more important pollinators of *S. lateriflorum* (PPE 220.1 vs. 121.1). Plant’s floral structure of *S. lateriflorum* also plays a key role in Halictid bee’s efficiency in pollination by allowing more pollen contact with the bee’s bodies.

Layek *et al*. (2022) reported that honeybees which are mixed foragers have greater abundance and higher values of the single-visit pollination efficiency index (PEi). The value of the combined parameter ‘importance in pollination (PI)’ was thus also higher in mixed foragers.

5. Conclusion

The PIV and PII indicated that *A. florea* was the most efficient pollinator of Aster compared to *A. cerana indica.* This show *Apis* beesplay the crucial role in the reproductive success of Aster, *Callistephus chinensis* by enhancing the seed set of Aster. Other pollinators also contributed to Aster pollination but in lesser extent which means pollinator diversity conservation is crucial in aster. These findings provide valuable insights for optimizing pollination management in commercial Aster cultivation, promoting the use of effective pollinator species to improve crop yields. Bottom of Form

Disclaimer (Artificial intelligence)

We hereby declare that NO generative AI technologies have been used during the writing or editing of this manuscript.

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