



## RESEARCH ARTICLE - BEES

## Assessing the Effects of Site, Season, and Honey Bee Pollination on Fruit Set and Yield in Aonla (*Emblica officinalis*) Orchards

V. DEVA DHARSHINI<sup>1</sup>, K. SURESH<sup>2</sup>, B. USHA RANI<sup>3</sup>, B. SAAI VIGNESH<sup>3</sup>, A. ARUN<sup>4</sup>, R. PROMOTH KUMAR<sup>1</sup>, K. BALAJI<sup>5</sup>

1 - Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India

2 - ICAR – Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

3 - Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Madurai, Tamil Nadu, India

4 - Department of Entomology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

5 - Department of Agricultural Engineering, Sethu Institute of Technology, Virudhunagar, Tamil Nadu, India

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#### Corresponding author

V. Deva Dharshini  
 Department of Agricultural Entomology, Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU) Coimbatore – 641 003, Tamil Nadu, India  
 E-Mail: devadharshini.phdent2023@tnau.ac.in

### Abstract

Aonla is a nutrient-rich, underutilized fruit crop in which efficient pollination increases yield. The study was carried out at two different locations in 2023 to determine the abundance of insect visitation and the effect of bee pollination on the yield of Aonla. The relative abundance of insect visitor studies revealed 22 and 17 species of insects visiting the Aonla flowers at locations I and II, respectively. Insect visitors were abundant during the 1000 – 1200 h period of the study. At location I, 11 species were hymenoptera, four were diptera, one was coleoptera, and four were lepidoptera. In contrast, in location II, nine were hymenoptera, three were diptera, one was coleoptera, and five were lepidoptera. Among these insect visitors of the hymenopteran family Apidae, *Apis cerana indica* and *Tetragonula iridipennis* were recorded to be the most frequent. To evaluate the influence of bee pollination on fruit set and yield, *A. cerana indica* and *T. iridipennis* colonies were placed in two different Aonla ecosystems. A significant increase ( $p = 0.05$ ) in fruit set (8.53–19.59%), retention (39.86–58.46%), and an increase in fruit weight (8.93–34.57 g), yield per tree (9.79–37.13%) was observed in treated plots compared to control plots in both locations. It is concluded that four colonies of *T. iridipennis*/acre can improve pollination in Aonla flowers to a great extent.

### Introduction

India, despite its rich biodiversity, contributes only 5% to global pollination research, which remains fragmented across crops, regions, and pollinator guilds (Adit et al., 2024). Empirical studies highlight the critical role of pollinators such as *Apis dorsata* in enhancing coffee fruit set by 50% in Kodagu (Krishnan et al., 2012) and increased pomegranate yield from 15.3 to 24.9 kg per plant in Karnataka (Vijayakumar et al., 2024), floral enhancements in Tamil Nadu moringa

plantations boosted pollinator abundance by 50% and fruit set by 30% (Dhandapani et al., 2023). Beyond crops, diverse bee communities on *Vitex negundo* (Nath et al., 2025), floral specialization of *Tetralonia macroceps* (Udayakumar et al., 2023), and adaptive nesting of stingless bees (Sabatina et al., 2024) underscore the ecological breadth and resilience of wild pollinators, which consistently outperform managed honeybees. National policies, such as the Sweet Revolution and NMSA, focus narrowly on beekeeping, overlooking the conservation of wild pollinators (Venu et al., 2024).



Major threats include habitat loss, pesticides, monocropping, invasive species, and climate change, with the impacts of pesticides underscoring the need for Integrated Pest and Pollinator Management (Mukhtar & Shankar, 2023).

Pollination services are valued at INR 22.5 billion annually (Venu et al., 2024), but research gaps persist in terms of regional coverage and crop diversity. Much work has been conducted in India on pollination studies of commercial fruit crops; however, limited information is available on highly cultivated, underutilized fruits and medicinal plants, such as Aonla (Vadde et al., 2018). Aonla (*Emblica officinalis* Gaertn.) is an important yet underutilized fruit in tropical and subtropical Indian regions (Goyal et al., 2007), and pollination improvement can lead to increased yields. The annual production of fruits in India was 335 million tonnes during the 2020–2021 period (Government of India, 2021). In Tamil Nadu, Aonla is cultivated on an area of 7,300 ha with an annual production of 173,930 metric tons due to its high nutritional value (Government of India, 2022).

Aonla, also known as Indian gooseberry, is highly valued for its rich nutritional profile and notable medicinal properties. It is one of the richest natural sources of vitamin C, containing 200–900 mg per 100 grams of fresh fruit pulp (Agarwal & Chopra, 2004). The increasing appeal of healthy foods, alternative medicines, and herbal products has increased the demand for Aonla, including its fruits and byproducts. It is a pungent, cool, diuretic, and laxative; therefore, it is used to treat colds, stomach problems, chronic diarrhoea, dysentery, headaches, constipation, hepatomegaly, diabetes, bronchitis, jaundice, and fever (Tripathi et al., 2020).

Crop wise, it is a highly branched monoecious shrub or tree that reaches a height of 9–18 m. The leaves were linear-oblong, obtuse, almost sessile, and densely branched into thin branches. Small yellow-green flowers are compact clusters in the axils of lower leaves. Male flowers usually appear at the base of shoots, with female flowers above them. These shoots bear flowers between February and March, with some regions in South India experiencing two flowering cycles, February–March and June–July. The limited pollination and imbalanced sex ratio contribute to low fruit set and retention (Allemullah & Ram, 1990). The pollen viability is initially high, up to 60–65% and decreases thereafter. Preliminary research by Pathak (2003) detected self-incompatibility during Aonla flowering and fruit set studies. The number of female flowers varied from two to eight, and the stigma became receptive from the third day of anthesis, remaining so for 48 hours. The insufficient fruit set may be attributed to the high proportion of staminate flowers.

Pollinators play a crucial role in the reproduction of numerous fruit crops. Honeybees, butterflies, bumblebees, hoverflies, solitary bees, beetles, and moths are the pollinators that are visited most often (Ollerton et al., 2011). Hence, the use of pollinators, especially bees, is considered one of the most cost-effective and environmentally friendly methods for maximizing Aonla yield.

Pollination is an essential environmental service in both natural ecosystems and agriculture (Ricketts et al., 2008). Pollination studies of various fruit crops have shown that pollinator management significantly improves the qualitative and quantitative parameters of all varieties. An effective pollinator will visit flowers regularly, collecting and transferring pollen to the stigma in a single visit. Bees are the most efficient pollinators (Potts et al., 2010), and pollinate over 70% of cultivated plant species (Ricketts et al., 2008).

Using pollinators, particularly honeybees, is considered a cost-effective and environmentally friendly method to increase the production of crops that require cross-pollination. Moreover, the foraging performance of bees has been widely investigated by Bharath et al. (2020), who reported that climatic variables have a significant influence on them. Numerous studies have confirmed that yield levels can be improved by 50–60 percent in fruit crops (Bharath et al., 2020; Muniz et al., 2020; Deepika et al., 2021). However, a comprehensive investigation into the pollination ecology and honey bee-mediated fruit setting in underutilized crops such as Aonla is lacking. The present study was conducted to explore the contributions of Indian bees and stingless bee-mediated pollination to yield improvements in the Aonla ecosystem.

## Materials and Methods

### *To study the insect visitors of the Aonla ecosystem*

The floral visitors present in the Aonla ecosystem were observed during 2023 at two different locations, viz., Madurai (9°97' N and 78°20' E) and Virudhunagar Districts (9°55' N and 78°09' E) of Tamil Nadu, India. Insect floral visitors were collected using glass vials and sweep nets, and preserved in 70% ethanol for further identification (Vasiliev & Aibulatov, 2023). Labelling was performed based on the date and time of collection of the insect sample, location, crop, and collector's name (Fursov et al., 2023). The collected specimens were identified based on their reproductive organ morphometrics under the guidance of the taxonomist Dr. U. Amala, Scientist, NBAIR, Bangalore. A thorough double-check verification of identified specimens on the available classification-based information in the "Fauna of British India" by Tablot (1978) and Borror and DeLong (2005) was also performed. This information was confirmed using digital keys accessible on various online platforms, such as the Museum of the Department of Agri-Entomology at the University of Agriculture Faisalabad (UAF), and the Entomological Research Institute located on Jhang Road in Faisalabad (Ghani & Maalik, 2020).

### *Foraging Behavior and Relative Abundance of Insect Visitors*

Observations were made on the number of insects visiting the inflorescences of Aonla for 5 minutes at two-hour intervals from 06:00 to 18:00. Five randomly selected Aonla trees were observed for 20 non-consecutive days of the flowering period from February to April 2023 (Season 1) and

June to August 2023 (Season 2). The relative abundance of species was calculated as a percentage by dividing the number of particular species by the total number of species visiting the flowers at two-hour intervals from 06:00 to 18:00 (Bakshi et al., 2018).

#### Meteorological Data

Maximum and minimum temperatures ( $^{\circ}\text{C}$ ), sunshine hours ( $\text{h day}^{-1}$ ), relative humidity (%), and the number of rainy days were recorded from the Automatic Weather Station located within a 700 m radius of the experimental fields. The number of observed foragers was correlated with the meteorological data of the corresponding days.

#### Honey Bee Pollination on the Fruit Set and Yield of Aonla

Two Field experiments were conducted at two different locations, Madurai ( $9^{\circ}97'$  N and  $78^{\circ}20'$  E) and Virudhunagar District ( $9^{\circ}55'$  N and  $78^{\circ}09'$  E) of Tamil Nadu, India, to study the influence of honey bee pollination on the fruit set and yield of Aonla (22 years old Chakkaiyah variety) during the two Aonla seasons of 2023. The experiment employed a Randomized Block Design with six treatments and three replications in a three-acre field, with a  $3 \times 3$  m spacing (Srinivasan et al., 2021).

#### Treatment Details

- T1** Two colonies of *Apis cerana indica*/acre
- T2** Four colonies of *Apis cerana indica*/acre
- T3** Two colonies of *Tetragonula iridipennis*/acre
- T4** Four colonies of *Tetragonula iridipennis*/acre
- T5** Pollinator exclusion (butter paper-covered inflorescence for five inflorescences per tree)
- T6** Aonla orchard without bee colonies (Control)

Honey bee colonies with uniform strength were selected for treatment assignment. Only healthy, disease-free, queen-right colonies with an actively laying queen were selected. Colonies were equalized by adjusting the adult bee population to 10 frames covered with bees, six frames of brood, and four empty frames in the super chamber. Standardization was completed 10 days prior to placement, allowing colonies to stabilize their foraging force and minimize variability in field performance.

Hives of Indian and stingless bees were placed in the Aonla orchard at approximately 10 percent flowering. After the installation of honeybee colonies in the field, chemical control measures were not taken. For the pollinator exclusion treatment, butter paper covers ( $35 \text{ cm} \times 35 \text{ cm}$ ) were tied to the panicles, and adequate perforations were made. No honeybee colonies were kept as the control group (Srinivasan et al., 2021).

#### Quantitative and Yield Parameters of Aonla

In each replication of the treatment, random observations were made from four selected trees, with five panicles per

tree, for the quantitative parameters of Aonla. Varied color ribbons, such as red, violet, blue, pink, yellow, and dark pink, were used to tie the panicles of the T1, T2, T3, T4, T5, and T6 treatments, respectively (Muniz et al., 2020). Data on fruit set percentage at the initial stage, fruit retention percentage at the harvest stage, fruit drop percentage from the initial fruit set to the harvest stage, fruit weight at the complete ripening stage, and yield ( $\text{kg/tree}$ ) data at the harvest stage were recorded (Mishra et al., 2023).

#### Statistical analysis

Data on insect visitor abundance were subjected to a square root transformation prior to analysis. Relative abundance was expressed as the mean number of individuals per inflorescence per 5 minutes. Differences in insect visitor abundance among treatments were analyzed using a one-way ANOVA, with treatment considered as a fixed factor and replication as a random factor. Where significant differences were detected ( $p < 0.05$ ), post-hoc comparisons of means were conducted using the DMRT at the 5% probability level.

To examine the influence of abiotic variables on the foraging activity of *Apis cerana indica* and *Tetragonula iridipennis*, multiple linear regression analyses were performed. Foraging activity (dependent variable) was regressed against maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, number of rainy days, and sunshine hours (independent variables, treated as continuous covariates). Models were constructed using the standard entry method, and the resulting regression equations and coefficients of determination ( $R^2$ ) were used to quantify the proportion of variation in foraging activity explained by the combined abiotic factors. In addition to the multiple regression models, simple linear regressions were performed for each abiotic factor individually against foraging activity to visualize pairwise relationships. Scatter plots with regression lines, corresponding equations, and  $R^2$  values were generated for each species at both locations.

Data on fruit set, fruit drop, fruit retention, fruit weight, and yield were analyzed using a three-factor factorial ANOVA with *season*, *location*, and *treatment* as fixed factors, and *replications* as random factors. Main effects as well as interactions among factors were evaluated. ANOVA assumptions of normality and homogeneity of variance were verified by Levene's test prior to analysis. Where significant differences were detected ( $p < 0.05$ ), post hoc mean comparisons were performed using both the Least Significant Difference (LSD) test. All statistical analyses were carried out using IBM SPSS Statistics v. 31.

#### Results

##### Relative Abundance of Insect Floral Visitors in Aonla Ecosystem

##### Location I

Among the 22 species recorded, 13 species (59.09%) belonged to hymenoptera, four species (18.18%) were dipterans,

one species (4.54%) was coleoptera, and four species (18.18%) were lepidopterans. Among hymenopterans, six belonged to the Apidae family and seven belonged to non-Apidae families, including Xylocopidae, Specidae, and Vespidae. Four species of diptera were identified in the families Muscidae, Sarcophagidae, and Syrphidae. One coleopteran species was identified as belonging to the Coccinellidae family, and nine lepidopteran species from the Erebidae, Lycaenidae, Pieridae, and Nymphalidae families were recorded.

The highest recorded insect abundance was *T. iridipennis* accounting for 18.80 - 33.31% in Season 1 and 21.29 - 30.00% in Season 2, followed by *A. cerana indica* with 14.73% - 23.22% in the first Season and 16.54 - 20.90% in the following Season, whereas *X. violaceae* with 0.00 to 0.83% was the least abundant in the February - April flowering period and 0.00 - 0.95% in the - June-July fowering period (Fig 1).

## Location II

The 17 species recorded in location II also belonged to the orders Hymenoptera, Diptera, Coleoptera, and Lepidoptera. Nine species (52.94%) were Hymenoptera, three (17.64%) were Diptera, one (5.88%) belonged to Coleoptera, and the remaining four (23.52%) were Lepidoptera. During the whole day, *T. iridipennis* (19.74% - 42.65% in Season 1 and 23.26 - 34.58% in Season 2) was relatively abundant, followed by *A. cerana indica* (19.81% - 25.32% in Season 1 and 17.34 - 28.20% in Season 2), and *A. dorsata* (5.70% - 10.52% in the first Season and 6.09 - 11.65% in the next Season). The minimum abundance of insects observed was *D. conoidium* (0 - 1.91% in Season 1 and 0.30 - 2.28% in Season 2) (Fig 1).

Since *T. iridipennis* and *A. cerana indica* peaked in visiting the Aonla flowers, an experiment was conducted to estimate the efficacy of pollination by primary pollinators in the Aonla ecosystem by framing treatments with various numbers of bee colonies.

## Influence of abiotic variables on the foraging behavior of honey bees

### Location I

The variations in abiotic factors like maximum temperature of 22.84% ( $R^2 = 0.2284$ ,  $p = 0.04$ ), minimum temperature (1.06%;  $p = 0.04$ ), morning (29.06%,  $p = 0.06$ ), evening relative humidity (58.13%,  $p = 0.09$ ), sunshine hours (43.21%,  $p = 0.09$ ), and rainy days (36.08%,  $p = 0.09$ ) resulted in the fluctuations of foraging activity of *A. cerana indica*. The foraging activity of *T. iridipennis* showed 9.97% ( $p = 0.07$ ), 6.84% ( $p = 0.05$ ), 7.31% ( $p = 0.01$ ), 1.86% ( $p = 0.07$ ), 1.52% ( $p = 0.05$ ), and 8.31% (0.01) variations in maximum temperature, minimum temperature, morning and evening relative humidity, sunshine hours, and rainy days, respectively (Fig 2a).

The coefficient of variation ( $R^2$ ) for the regression equation indicated that there was 67.65 percent ( $R^2 = 0.6765$ ) variation in the foraging activity of *A. cerana indica* due to abiotic factors. It was fitted in the multiple regression equation  $Y = 0.25 X_1 - 0.27 X_2 - 0.03 X_3 - 0.01 X_4 + 0.07 X_5 + 0.01 X_6$ . Similarly, a 62.55% variation in foraging activity was observed for *T. iridipennis* due to abiotic factors, and the fitted  $Y = 0.1537 X_1 - 0.4123 X_2 - 0.1642 X_3 - 0.00209 X_4 - 1.0938 X_5 - 0.1482 X_6 + 31.0646$  multiple regression equation line (Table 1).

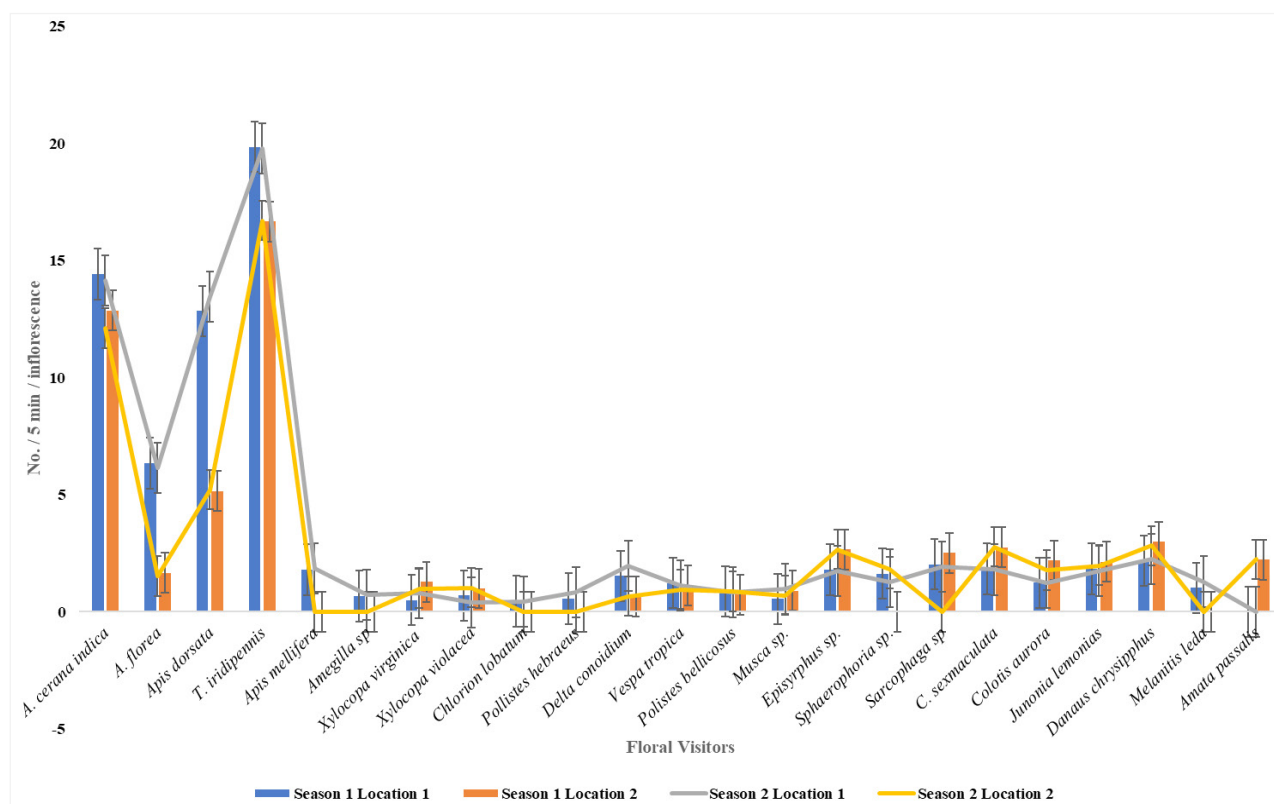
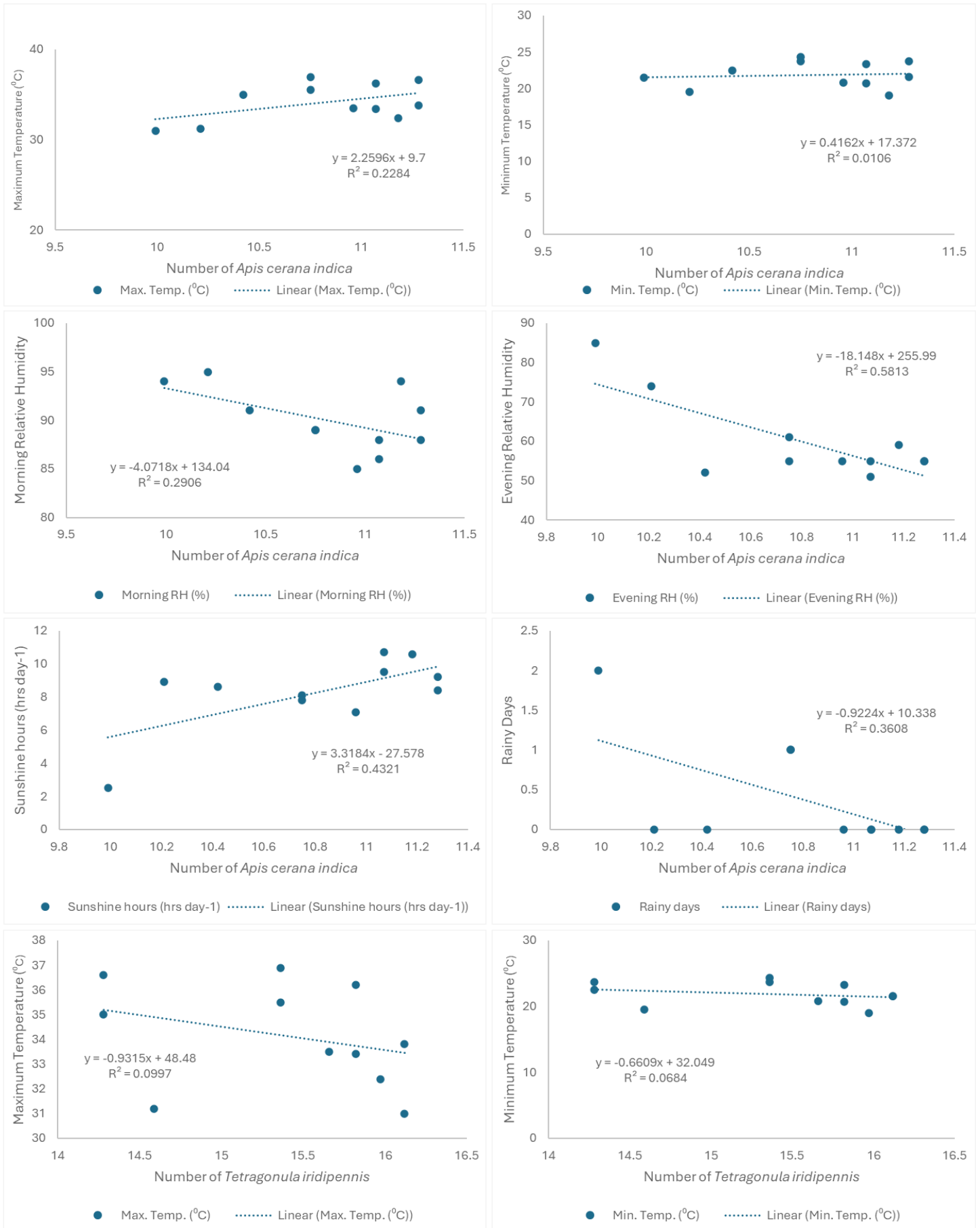
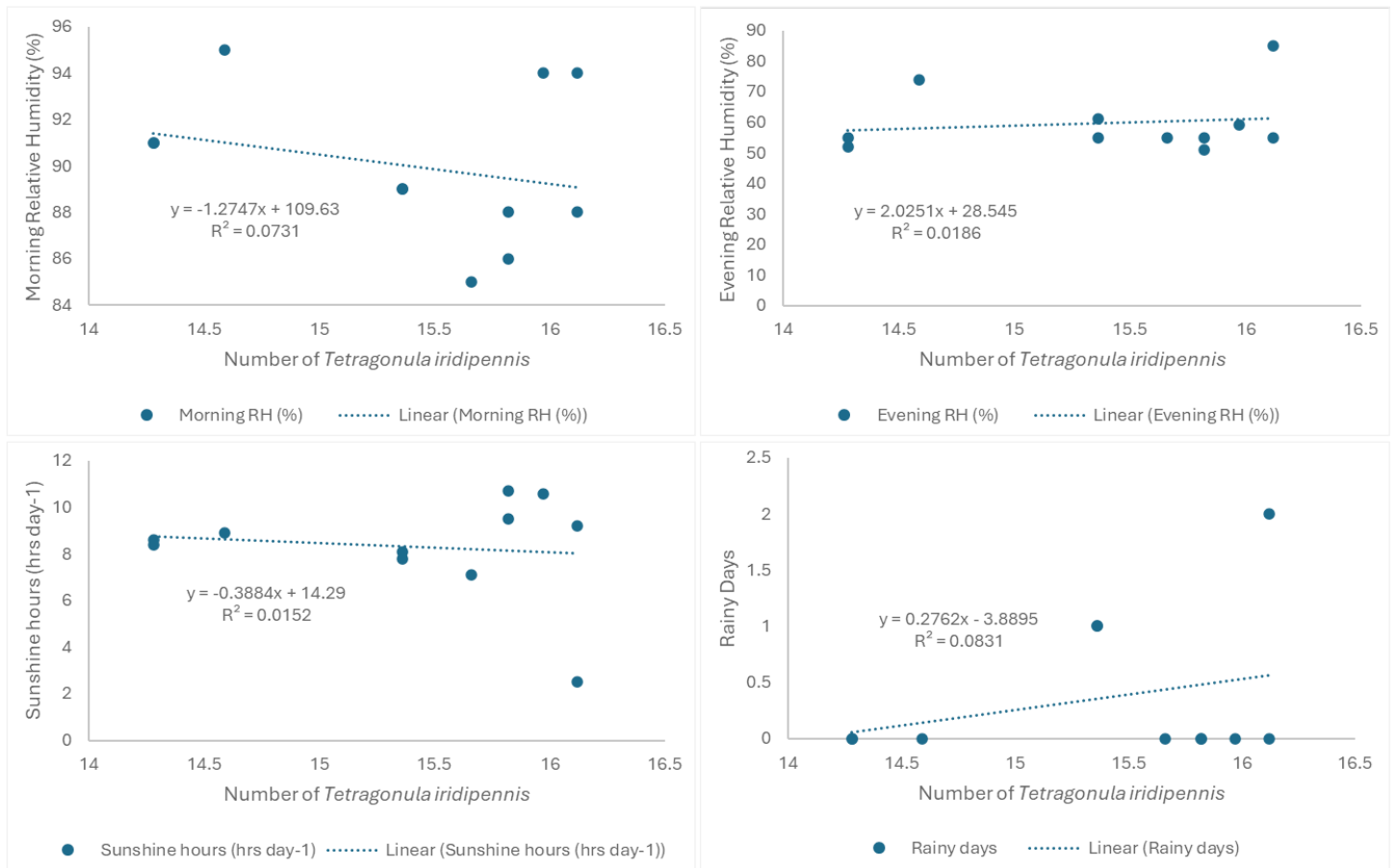


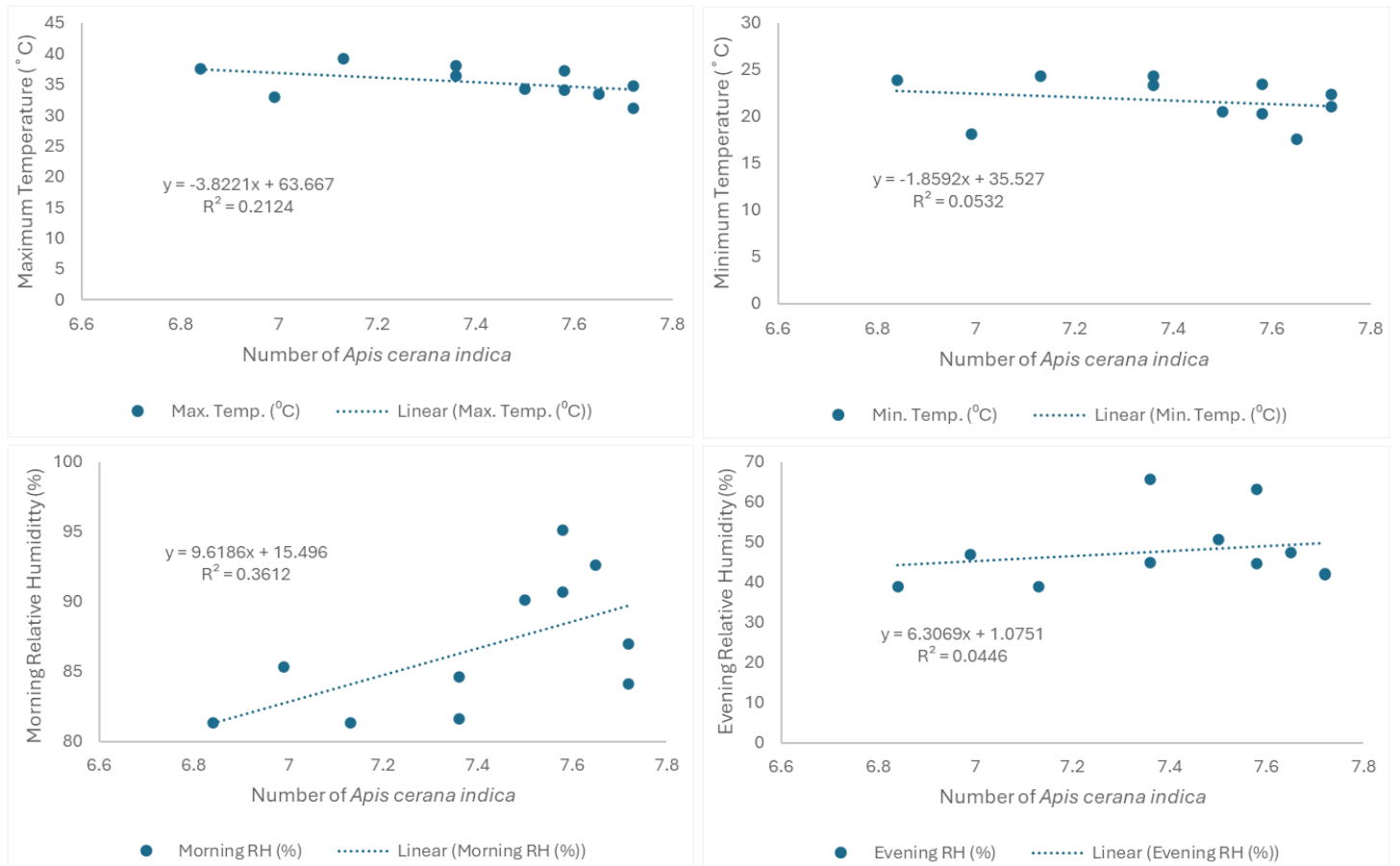
Fig 1. Relative Abundance of Insect Floral Visitors in Aonla Ecosystem.



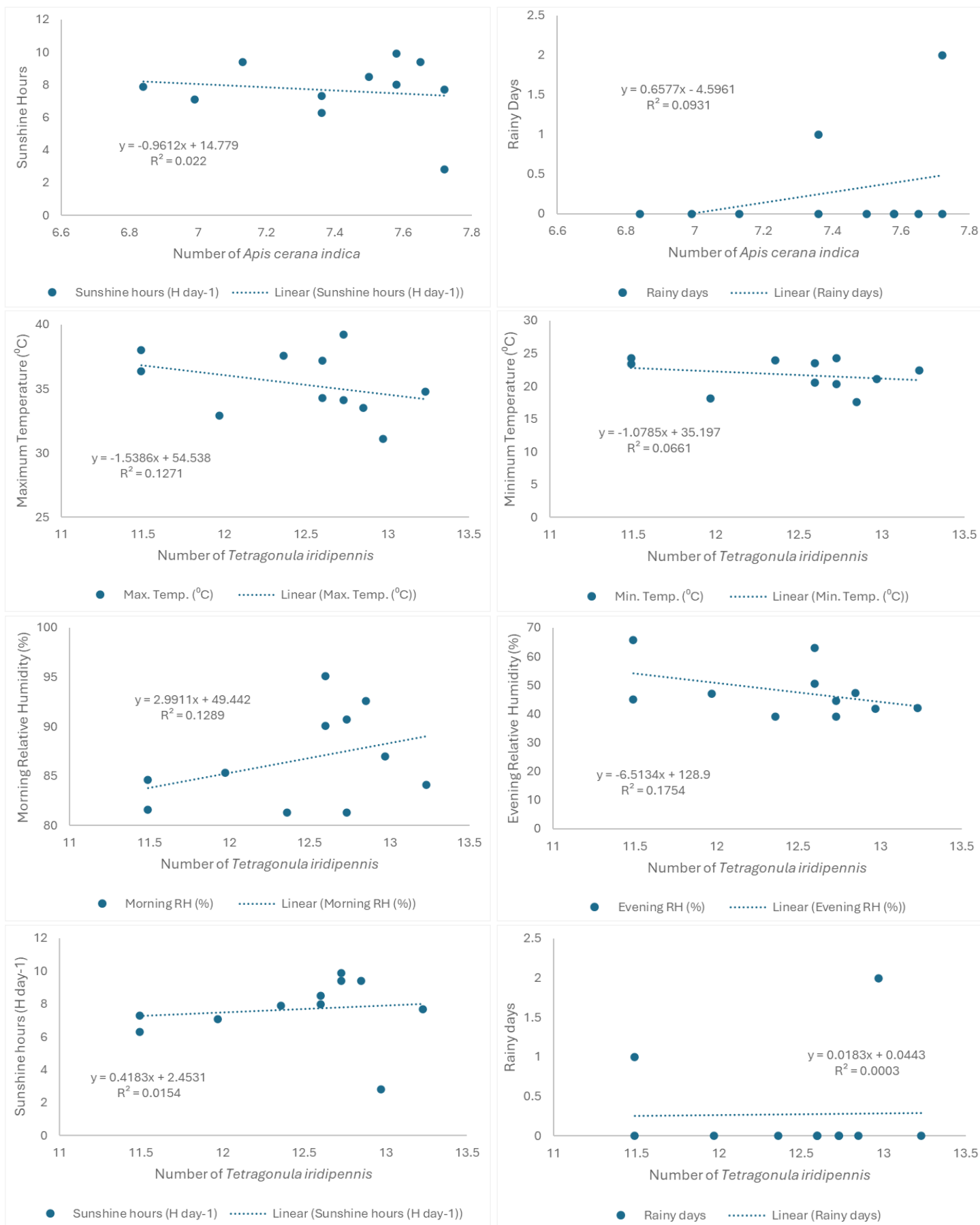
**Fig 2a.** Influence of abiotic variables on foraging behaviour at Location I.



**Fig 2a.** Influence of abiotic variables on foraging behaviour at Location I. (Continuation)



**Fig 2b.** Influence of abiotic variables on foraging behaviour at Location II.



**Fig 2b.** Influence of abiotic variables on foraging behaviour at Location II. (Continuation)

## Location II

The coefficients of determination ( $R^2$ ) for variations in the foraging activity of *A. cerana indica* in Orchard, RRS Aruppukottai due to the influence of abiotic factors were 21.24% ( $p = 0.01$ ; maximum temperature), 5.32% ( $p = 0.01$ ; minimum temperature), 36.12% ( $p = 0.08$ ; morning), 4.46% ( $p = 0.04$ ; evening relative humidity), 2.2% ( $p = 0.02$ ; sunshine hours), and 9.31% ( $p = 0.03$ ; rainy days). The foraging activity of *T. iridipennis* was influenced by variations in maximum temperature (12.71%,  $p = 0.05$ ), minimum temperature (6.61%,  $p = 0.02$ ), morning (12.89%,  $p = 0.01$ ), evening relative humidity (17.54%,  $p = 0.01$ ), sunshine hours (1.54%,  $p = 0.06$ ), and rainy days (0.03%,  $p = 0.02$ ) to a considerable extent (Fig 2b).

Multiple regression analysis revealed that there was a 73.71% ( $R^2 = 0.7371$ ) variation in the foraging activity of *A. cerana indica* due to abiotic factors and fitted to the equation  $Y = -0.2088 X_1 + 0.1563 X_2 + 0.0091 X_3 - 0.0159 X_4 - 0.3584 X_5 + 0.2030 X_6 + 8.175$ . The coefficient of variation ( $R^2$ ) for the regression equation indicated that there were 78.32% variations in the foraging activity of *T. iridipennis* due to the influence of abiotic factors and fitted in equation  $Y = -0.1488 X_1 - 0.2219 X_2 + 0.1304 X_3 - 0.071 X_4 - 0.6943 X_5 - 0.1196 X_6 + 6.1032$  (Table 1).

## Influence of honey bee pollination on quantitative and yield parameters of Aonla

### Fruit Set Percentage

The three factor factorial ANOVA revealed that fruit set percentage was significantly influenced by location (Factor B;  $F = 10.47$ ,  $p = 0.0023$ ) and treatment (Factor C;  $F = 1056.60$ ,  $p < 0.001$ ), whereas the effects of season (Factor A;  $F = 0.0517$ ,  $P = 0.8212$ ) and all interaction effects  $A \times B$  ( $F = 0.0269$ ,  $p = 0.8705$ ),  $A \times C$  ( $F = 0.0578$ ,  $p = 0.9977$ ),  $B \times C$  ( $F = 1.2681$ ,  $p = 0.2938$ ), and  $A \times B \times C$  ( $F = 0.0692$ ,  $p = 0.9965$ ) were not significant ( $p > 0.05$ ) (Table 2). According to LSD comparisons between the two locations, Location 2 had a significantly higher fruit set (62.76%) than Location 1 (60.29%). Moreover, among the treatments, substantial variation was observed, with T4 (77.49%) showing the highest fruit set, followed by treatments T1 (74.9%) and T2 (75.67%) that were statistically on par. T3 (74.02%) also recorded moderate fruit set, whereas T6 (67.05%) showed the lowest values among effective treatments. T5 failed to induce any fruit set (0.00%), which was significantly lower than all other treatments ( $p < 0.05$ ) (Fig 3).

**Table 1.** Regression analysis on the influence of abiotic variables on the foraging behaviour of honey bees.

Locations	Species	Multiple Linear Regression Equation Value	R <sup>2</sup> Value
AC & RI, Madurai	<i>A. cerana indica</i>	$Y = 0.2545 X_1^* - 0.2657^* X_2 - 0.0304 X_3 - 0.0064 X_4 + 0.0708 X_5 + 0.0109 X_6 + 10.9438^*$	0.6765
	<i>T. iridipennis</i>	$Y = 0.1537 X_1 - 0.4123 X_2 - 0.1642^* X_3 - 0.0209 X_4 - 1.0938^* X_5 - 0.1482 X_6 + 31.0646^*$	0.6255
RRS, Aruppukottai	<i>A. cerana indica</i>	$Y = -0.2088^* X_1 + 0.1563^* X_2 + 0.0091 X_3 - 0.0159^* X_4 - 0.3584^* X_5 + 0.2030^* X_6 + 8.175^*$	0.7371
	<i>T. iridipennis</i>	$Y = -0.1488 X_1 - 0.2219 X_2^* + 0.1304^{**} X_3 - 0.071^{**} X_4 - 0.6943^* X_5 - 0.1196 X_6 + 6.1032^*$	0.7832

X1 – Maximum Temperature, X2 – Minimum Temperature, X3 – Relative humidity% (Morning hours), X4 – Relative Humidity% (Evening hours), X5 – Rainy days, X6 – Sunshine Hours \*\*significance at 0.01 level (2-tailed test), \*significant at the 0.05 level (2-tailed test)

**Table 2.** Three-way ANOVA showing the effects of Season (Factor A), Location (Factor B), and Treatment (Factor C) on fruit set percentage.

	df	SS	MSS	F	Pr (>F)	Sig.	Factor B	
Replication	2	82.367	41.1835	3.9363	0.0264	*	L1	60.291 <sup>b</sup>
Factor A	1	0.5408	0.5408	0.0517	0.8212	NS	L2	62.758 <sup>a</sup>
Factor B	1	109.52	109.52	10.4679	0.0023	**	Factor C	
Factor C	5	55273.2934	11054.6587	1056.5991	0	***	T1	74.9 <sup>ab</sup>
A X B	1	0.2812	0.2812	0.0269	0.8705	NS	T2	75.67 <sup>ab</sup>
A X C	5	3.0222	0.6044	0.0578	0.9977	NS	T3	74.02 <sup>b</sup>
B X C	5	66.3377	13.2675	1.2681	0.2938	NS	T4	77.49 <sup>a</sup>
A X B X C	5	3.618	0.7236	0.0692	0.9965	NS	T5	0.00 <sup>d</sup>
Error	46	481.2746	10.4625	NA	NA	NA	T6	67.05 <sup>c</sup>

\*\*\*significant at 0.001 level; \*\*significant at 0.01 level; \*significant at 0.05 level; NS - not significant at 0.05 level; NA -not applicable. The multiple comparison test (LSD) is performed only for those effects that are significant. In a column, means followed by same letter(s) are on par by DMRT ( $p = 0.05$ ).

### Fruit Drop Percentage

The fruit drop percentage was significantly influenced by location (Factor B;  $F = 8.41, p = 0.0057$ ) and treatment (Factor C;  $F = 1066.86, p < 0.001$ ), whereas the effects of season (Factor A;  $F = 0.1378, p = 0.7122$ ) and all interaction effects such as  $A \times B$  ( $F = 0.2257, p = 0.6370$ ),  $A \times C$  ( $F = 0.1065, p = 0.9903$ ),  $B \times C$  ( $F = 1.2176, p = 0.3161$ ), and  $A \times B \times C$  ( $F = 0.1775, p = 0.9697$ ) were not significant (Table 3).

A multiple comparison test revealed that location 1 exhibited significantly higher fruit drop (63.34%) than location 2 (61.11%). Among the treatments, substantial differences were observed in drop percent depicting T6 (81.95%) as the highest fruit drop, which was significantly greater than all other treatments ( $p < 0.05$ ) followed by T3 (77.51%), while T1 (72.64%), T2 (70.30%), and T4 (70.96%) had a statistically on par with comparatively lower fruit drop (Fig 4).

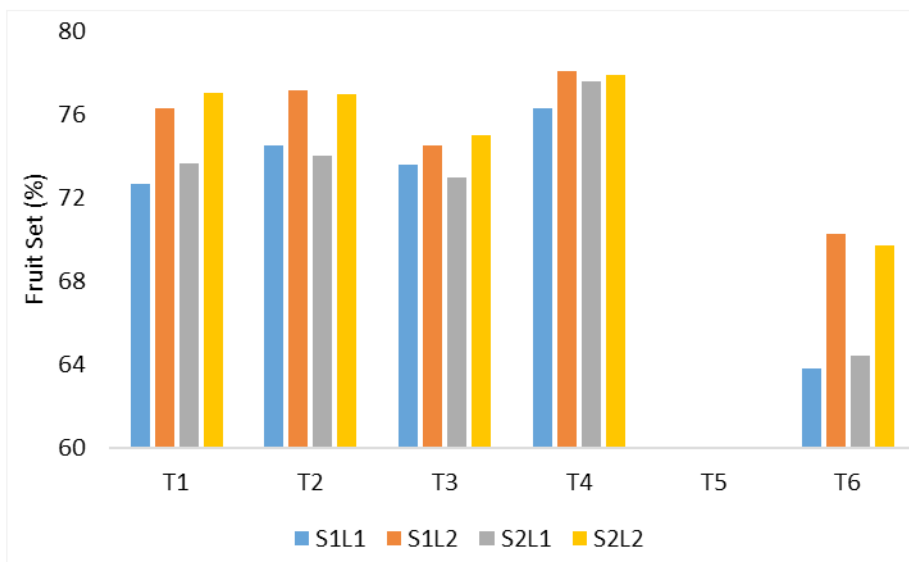


Fig 3. Effect of Season (Factor A), Location (Factor B), and Treatment (Factor C) on fruit set percentage.

Table 3. Three-way ANOVA showing the effects of Season (Factor A), Location (Factor B), and Treatment (Factor C) on fruit drop percentage.

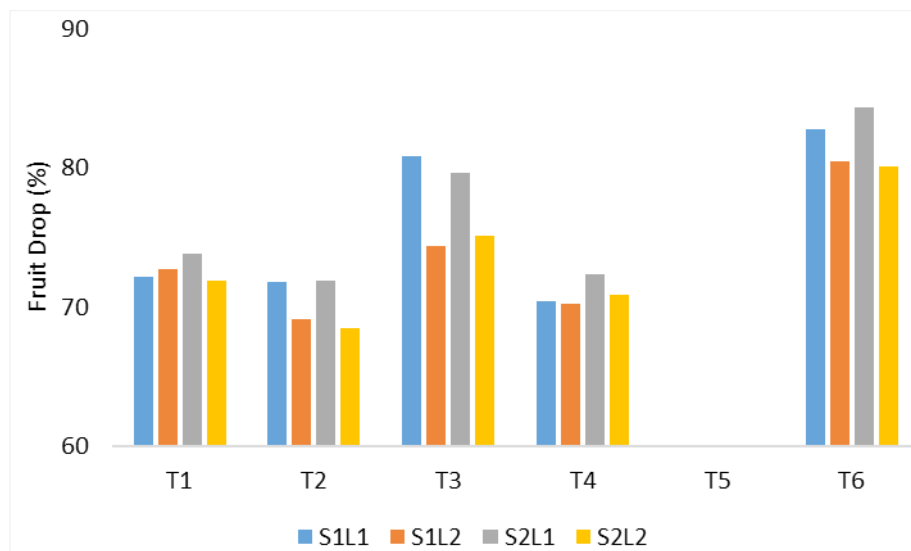
	df	SS	MSS	F	Pr (>F)	Sig.	Factor B	
Replication	2	89.0195	44.5097	4.1702	0.0217	*	L1	63.34 <sup>a</sup>
Factor A	1	1.4706	1.4706	0.1378	0.7122	NS	L2	61.11 <sup>b</sup>
Factor B	1	89.713	89.713	8.4054	0.0057	**		<b>Factor C</b>
Factor C	5	56934.1375	11386.8275	1066.861	0	***	T1	72.640 <sup>c</sup>
A X B	1	2.409	2.409	0.2257	0.637	NS	T2	70.302 <sup>c</sup>
A X C	5	5.6825	1.1365	0.1065	0.9903	NS	T3	77.513 <sup>b</sup>
B X C	5	64.9771	12.9954	1.2176	0.3161	NS	T4	70.957 <sup>c</sup>
A X B X C	5	9.4751	1.895	0.1775	0.9697	NS	T5	0.000 <sup>d</sup>
Error	46	490.9675	10.6732	NA	NA	NA	T6	81.945 <sup>a</sup>

\*\*\*significant at 0.001 level; \*\*significant at 0.01 level; \*significant at 0.05 level; NS - not significant at 0.05 level; Multiple comparison test (LSD) is performed only for those effects which are significant. In a column, means followed by same letter(s) are on par by DMRT ( $p = 0.05$ ).

### Fruit Retention Percentage

The fruit retention percentage was significantly influenced by season (Factor A;  $F = 4.09, p = 0.049$ ), location (Factor B;  $F = 6.64, p = 0.0132$ ), and treatment (Factor C;  $F = 1135.26, p < 0.001$ ). A significant interaction was also observed between season  $\times$  location ( $A \times B; F = 6.03, p = 0.0179$ ), whereas the other interactions such as  $A \times C$  ( $F = 0.47, p = 0.7962$ ),  $B \times C$  ( $F = 1.74, p = 0.1452$ ), and  $A \times B \times C$  ( $F = 0.74, p = 0.5972$ )

were not significant (Table 4). According to LSD comparisons, Season 1 (22.17%) recorded a significantly higher fruit retention than Season 2 (21.61%). Between locations, L2 (22.24%) exhibited greater fruit retention compared to L1 (21.53%). A significant interaction of season  $\times$  location revealed that fruit retention was lowest under Season 2  $\times$  Location 1 (20.91%), while the other season  $\times$  location combinations (22.15-22.30%) had a statistically comparable retention percentage (Table 5).



**Fig 4.** Effect of Season (Factor A), Location (Factor B), and Treatment (Factor C) on fruit drop percentage.

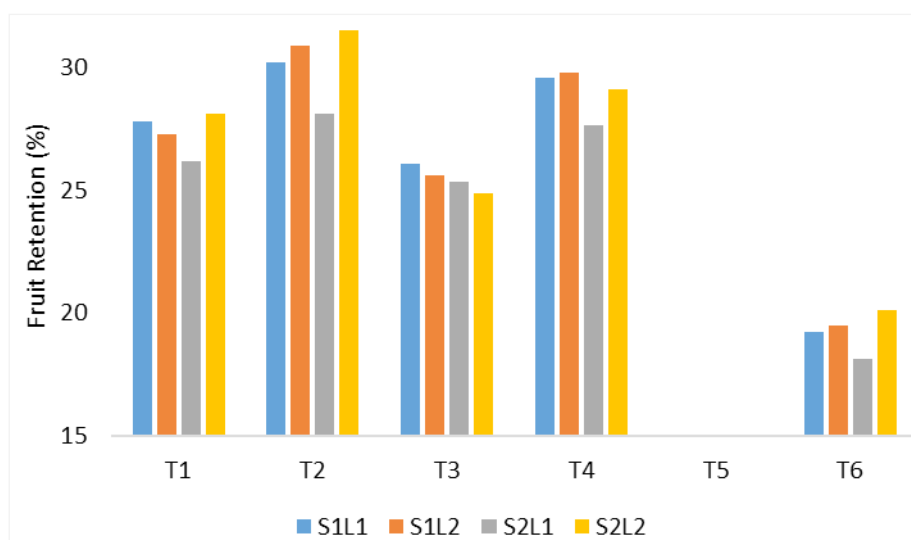
**Table 4.** Three-way ANOVA showing the effects of Season (Factor A), Location (Factor B), and Treatment (Factor C) on fruit retention percentage.

	df	SS	MSS	F	Pr (>F)	Sig.
<b>Replication</b>	2	9.4518	4.7259	3.4432	0.0404	*
<b>Factor A</b>	1	5.6112	5.6112	4.0883	0.049	*
<b>Factor B</b>	1	9.1165	9.1165	6.6421	0.0132	*
<b>Factor C</b>	5	7790.9224	1558.1845	1135.2645	0	***
<b>A X B</b>	1	8.2825	8.2825	6.0344	0.0179	*
<b>A X C</b>	5	3.2294	0.6459	0.4706	0.7962	NS
<b>B X C</b>	5	11.9181	2.3836	1.7367	0.1452	NS
<b>A X B X C</b>	5	5.081	1.0162	0.7404	0.5972	NS
<b>Error</b>	46	63.1364	1.3725	NA	NA	NA

\*\*\*significant at 0.001 level; \*\*significant at 0.01 level; \*significant at 0.05 level; NS - not significant at 0.05 level; Multiple comparison test (LSD) is performed only for those effects which are significant.

Among the treatments, significant variation was observed in T2 (30.20%), which achieved the highest fruit retention, followed by T4 (29.04%), both of which were significantly superior to other treatments, followed by T1 (27.36%), T3

(25.49%), and T6 (19.24%), which recorded comparatively lower retention percent. In contrast, T5 (0.00%) failed to retain any fruit, which was significantly lower than all other treatments ( $p < 0.05$ ) (Fig 5).



**Fig 5.** Effect of Season (Factor A), Location (Factor B), and Treatment (Factor C) on fruit retention percentage.

**Fruit Weight (g)**

Only different treatment (Factor C;  $F = 1095.19$ ,  $p < 0.001$ ) influenced the variations in fruit weight, while the effects of season (Factor A;  $F = 0.093$ ,  $p = 0.7618$ ) and location (Factor B;  $F = 0.120$ ,  $p = 0.7310$ ), as well as all interaction effects such as  $A \times B$  ( $F = 0.0047$ ,  $p = 0.9454$ ),  $A \times C$  ( $F = 0.0515$ ,  $p = 0.9982$ ),  $B \times C$  ( $F = 0.7754$ ,  $p = 0.5724$ ), and  $A \times B \times C$  ( $F = 0.0122$ ,  $p = 0.9999$ ) were not significant (Table 6). Multiple comparison test (LSD) revealed that substantial differences were observed among treatments. T4 (167.72 g)

and T2 (164.50 g) had the highest fruit weights, followed by T1 (145.88 g) and T3 (151.21 g), which showed moderate weights and were statistically on par. T6 (137.37 g) had the lowest ten fruit weight among the effective treatments, while T5 (0.00 g) failed to produce any fruit weight, being significantly inferior to all other treatments ( $p < 0.05$ ) (Fig 6).

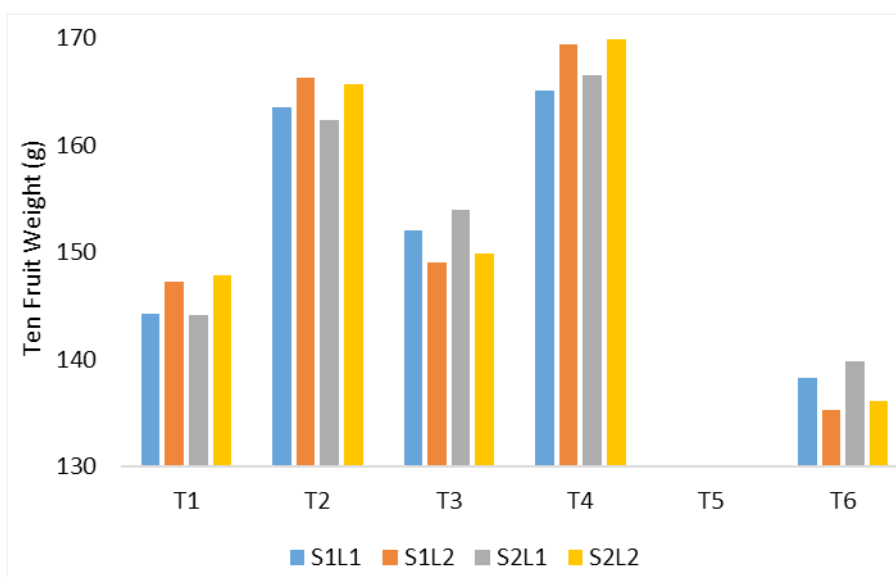
**Yield (Kg/tree)**

Significant variation in yield per tree was observed across locations (Factor B;  $F = 19.12$ ,  $p < 0.001$ ) and treatments (Factor C;  $F = 1246.87$ ,  $p < 0.001$ ) while the effects of season (Factor A;  $F = 0.0214$ ,  $p = 0.8844$ ) and all interaction effects such as  $A \times B$  ( $F = 0.0266$ ,  $p = 0.8711$ ),  $A \times C$  ( $F = 0.2182$ ,  $p = 0.9530$ ),  $B \times C$  ( $F = 1.3911$ ,  $p = 0.2453$ ), and  $A \times B \times C$  ( $F = 0.0347$ ,  $p = 0.9993$ ) were not significant (Table 7). Post hoc

**Table 5.** Main Effects and Interaction Effects of Season, Location, and Treatment on Fruit Retention (%).

Factor A	
Season 1	22.167 <sup>a</sup>
Season 2	21.608 <sup>b</sup>
Factor B	
Location 1	21.532 <sup>b</sup>
Location 2	22.243 <sup>a</sup>
Factor C	
T1	27.360 <sup>c</sup>
T2	30.198 <sup>a</sup>
T3	25.488 <sup>d</sup>
T4	29.043 <sup>b</sup>
T5	0.000 <sup>f</sup>
T6	19.238 <sup>c</sup>
Factor (AxB)	
1 X 1	22.150 <sup>a</sup>
1 X 2	22.183 <sup>a</sup>
2 X 1	20.913 <sup>b</sup>
2 X 2	22.303 <sup>a</sup>

In a column, means followed by same letter(s) are on par by DMRT ( $p = 0.05$ ).



**Fig 6.** Effect of Season (Factor A), Location (Factor B), and Treatment (Factor C) on ten fruit weight in grams.

**Table 6.** Three-way ANOVA showing the effects of Season (Factor A), Location (Factor B), and Treatment (Factor C) on the ten fruit weights in grams.

	df	SS	MSS	F	Pr (>F)	Sig.	Factor C	
<b>Replication</b>	2	331.4166	165.7083	3.736	0.0314	*	T1	145.884 <sup>b</sup>
<b>Factor A</b>	1	4.1242	4.1242	0.093	0.7618	NS	T2	164.502 <sup>a</sup>
<b>Factor B</b>	1	5.3073	5.3073	0.1197	0.731	NS	T3	151.210 <sup>b</sup>
<b>Factor C</b>	5	242882.5172	48576.5034	1095.1901	0	***	T4	167.720 <sup>a</sup>
<b>A X B</b>	1	0.21	0.21	0.0047	0.9454	NS	T5	0.000 <sup>d</sup>
<b>A X C</b>	5	11.4266	2.2853	0.0515	0.9982	NS	T6	137.373 <sup>c</sup>
<b>B X C</b>	5	171.9719	34.3944	0.7754	0.5724	NS		
<b>A X B X C</b>	5	2.711	0.5422	0.0122	0.9999	NS		
<b>Error</b>	46	2040.3025	44.3544	NA	NA	NA		

\*\*\*significant at 0.001 level; \*\*significant at 0.01 level; \*significant at 0.05 level; NS - not significant at 0.05 level; Multiple comparison test (LSD) is performed only for those effects which are significant; In a column, means followed by the same letter(s) are on par by DMRT ( $p = 0.05$ ).

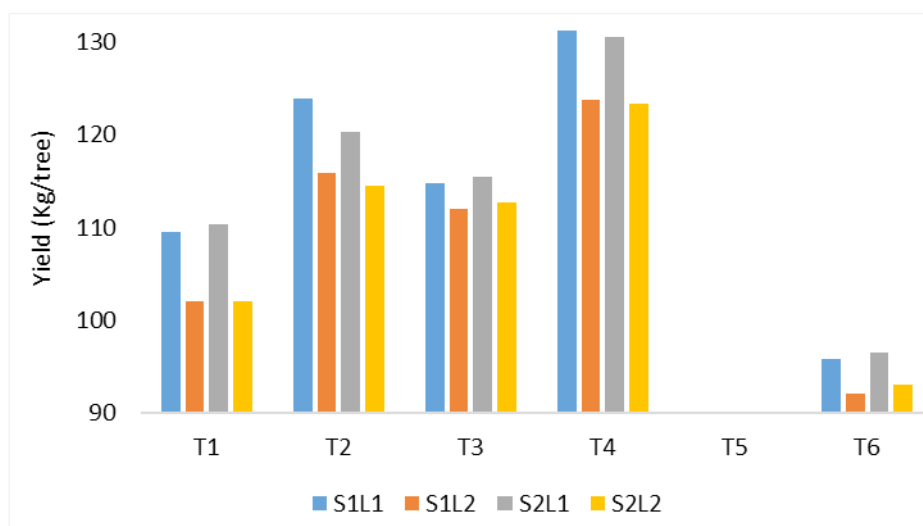
comparisons revealed that Location 1 (95.69 kg/tree) yielded significantly more than Location 2 (90.93 kg/tree). Among the treatments, T4 (127.26 kg/tree) produced the maximum yield, followed by T2 (118.64 kg/tree), T3 (113.70 kg/tree), and T1

(105.94 kg/tree), whereas T6 (94.29 kg/tree) produced the lowest among the effective treatments. In contrast, T5 (0.00 kg/tree) failed to produce any yield, which was significantly inferior to all others ( $p < 0.05$ ) (Fig 7).

**Table 7.** Three-way ANOVA showing the effects of Season (Factor A), Location (Factor B), and Treatment (Factor C) on yield (kg/tree).

	df	SS	MSS	F	Pr (>F)	Sig.	Factor B	Factor C
Replication	2	124.8326	62.4163	2.9274	0.0636	NS	L1	95.685 <sup>a</sup>
Factor A	1	0.4556	0.4556	0.0214	0.8844	NS	L2	90.926 <sup>b</sup>
Factor B	1	407.7054	407.7054	19.1217	1e-04	***		
Factor C	5	132926.0899	26585.218	1246.8665	0	***	T1	105.940 <sup>d</sup>
A X B	1	0.5675	0.5675	0.0266	0.8711	NS	T2	118.642 <sup>b</sup>
A X C	5	23.26	4.652	0.2182	0.953	NS	T3	113.698 <sup>c</sup>
B X C	5	148.3067	29.6613	1.3911	0.2453	NS	T4	127.259 <sup>a</sup>
A X B X C	5	3.7025	0.7405	0.0347	0.9993	NS	T5	0.000 <sup>f</sup>
Error	46	980.7947	21.3216	NA	NA	NA	T6	94.293 <sup>c</sup>

\*\*\*significant at 0.001 level; \*\*significant at 0.01 level; \*significant at 0.05 level; NS - not significant at 0.05 level; Multiple comparison test (LSD) is performed only for those effects which are significant; In a column, means followed by the same letter(s) are on par by DMRT ( $p = 0.05$ ).



**Fig 7.** Effect of Season (Factor A), Location (Factor B), and Treatment (Factor C) on yield (kg tree<sup>-1</sup>).

## Discussion

Aonla has gained global recognition and widespread acceptance for its nutritional and functional properties (Tripathi et al., 2020). The limited pollination and imbalanced sex ratio contribute to low fruit set and retention (Allemullah & Ram, 1990). Preliminary research by Pathak (2003) detected self-incompatibility during Aonla flowering and fruit set studies. Pollination is a crucial environmental service in both natural ecosystems and agriculture (Ricketts et al., 2008). Pollination studies of various fruit crops have shown that pollinator management significantly improves the qualitative and quantitative parameters of all varieties. An effective

pollinator visits flowers repeatedly, collects pollen, and then deposits it onto the stigma in a single visit. Insect visitor studies on Aonla blossoms conducted by Singh et al. (2009) reported 28 arthropods, of which the hymenopteran family Apidae was documented as the predominant species. Singh et al. (2018) reported that Aonla blossoms were attracted to 31 and 28 arthropods in 2013 and 2014, respectively.

The relationship between insects and climatic variability plays a significant role in the type and abundance of insects visiting flowers. In our study, it was evident that the influence of abiotic factors led to a 67.65 – 73.71% change in the foraging activity of *A. cerana indica* and 62.55 – 78.32% variations in *T. iridipennis*.

Uncertainty in temperature, sunshine hours, relative humidity, and rainy days affects pollinator abundance (Bharath et al., 2020). Thermal constraints were the major factors influencing the foraging activity of honeybees. Polatto et al. (2014) reported that the foraging activities of bees depend on temperature and luminosity, rather than relative humidity and wind speed. This result also follows the findings of Alves et al. (2015), who found that the foraging activity of *A. mellifera* was highly influenced by the maximum temperature and lower relative humidity and accounted for a 46.90% increase in the foraging activity of bees. Joshi et al. (2015) have shown that minimum temperatures below 20 °C, rain, wind speed, and low light intensity negatively affect the foraging activity of bees. Asokan et al. (2022) investigated the impact of wind speed and relative humidity on the foraging activity of bees. Mohanty et al. (2023) found that rainfall had a negative correlation with the foraging activity of bees, and temperature had a significant positive correlation.

Multiple regression analysis performed by Bharath et al. (2020) in the same location as the study on the contribution of climatic parameters to bee foraging reported that maximum temperature ( $a = +0.92$ ) had a positive regression coefficient and minimum temperature ( $b = -4.99$ ), relative humidity ( $c = -0.14$ ), and rainfall ( $r = -0.06$ ) were negatively correlated with foragers. Similar results were reported by Hemalatha et al. (2018) in the Madurai District of Tamil Nadu, India. In contrast, Reddy et al. (2015) found that the maximum temperature had a negative effect on the number of bees moving out to forage ( $r = -0.72$ ,  $p = 0.01$ )

Studies carried out in the same agro-climatic zone as the present study on insect visitor abundance in bitter gourd ecosystems revealed hymenopterans as the major visitors, followed by lepidopterans and dipterans (Yogapriya et al., 2019). In contrast, in the sesame ecosystem, hymenopterans were the most abundant insects (Selvakumari et al., 2022). Furthermore, a study of coriander ecosystems revealed that Hymenoptera, followed by Lepidoptera, Diptera, Hemiptera, and Coleoptera, were the major visitors (Madhuvandhi et al., 2021). Twelve insects belonging to five different orders were documented in the Aonla orchard, where the order of abundance was Diptera > Hymenoptera > Coleoptera > Lepidoptera > Hemiptera (Vadde et al., 2018).

The peak abundance of insect visitors in the Aonla ecosystem was recorded between 10:00 and 12:00 during the study, and the minimum foraging activity was recorded between 06:00 and 08:00 during the study (Vadde et al., 2018). Another study by Sihag and Saini (2023) revealed that Aonla blossoms foraged by *Apis* sp. had the highest levels throughout the day compared with those foraged by non-*Apis* species. Similarly, significant pollinator activity in the mango ecosystem was recorded between 09:00 and 11:00 (9.75 pollinators), whereas minimal activity was noted from 14:00 to 17:00 (1.90 pollinators) (Deuri et al., 2018). The relative abundance of bees was higher during 11:00 – 12:00 and remained at a minimum from 14:00 to 16:00 in

a maize ecosystem (Painkra et al., 2021). This study was also supported by the results of Shivaramu et al. (2012) for *Nephelium lappacum* L., Vishwakarma & Singh (2017) in the mango ecosystem, and Joshi & Joshi (2010) in apple, who reported that the peak activity of hymenopteran foragers occurred in the morning hours rather than in the evening hours.

Of all the floral visitors recorded, *T. iridipennis*, *A. cerana indica*, and *A. dorsata* were recorded as abundant visitors to the Aonla ecosystem. This finding is in close agreement with previous studies in Aonla Orchard (Singh et al., 2009; Singh et al., 2018; Vadde et al., 2018; Sihag & Saini, 2023). Similar results were reported by Vanitha & Raviprasad (2019) in cashews, where hymenopterans such as *A. cerana indica*, *A. florea*, and *Tetragonula* sp. were recorded as the predominant floral visitors. *Apis cerana indica* accounts for approximately 46.46% of pollination in mango ecosystems (Deuri et al., 2018). The blossoms of cumin have been pollinated by Apidae (62.40%), followed by dipterans (27.50%) (Meena et al., 2018). In the mango ecosystem, fruit set increased up to 23.72% with the placement of Indian bee colonies (Deuri et al., 2018). Bhattacharya et al. (2004) reported that honeybees significantly increased the fruit set ratio compared to that of the control, which also recorded no fruit set in the pollination exclusion treatment. The use of honey bees significantly increased the fruit set ratio (41.00%) compared to that of the control (2.13%) in the mango ecosystem (Fajardo et al., 2008).

This finding was also in line with the results of Deepika et al. (2021), who reported that the orchard with four colonies of *A. cerana indica* and *T. iridipennis* per acre exhibited the highest fruit set and retention percentage per panicle (4.62 and 5.02 fruits/panicle).

The percentage of fruit retention of moringa in the two colonies per acre was 5.00% higher than that in the field with one colony per acre. The total fruits per tree (63.00), fruit length (63.20 cm), fruit girth (8.10 cm), individual fruit weight (111.20 g) and total fruit weight (7.00 kg/ tree) increased (13.90%) in two bee colonies/acre than in one bee colony per acre (7.60%) in moringa where the total fruits per tree (61.40), fruit length (59.20 cm), fruit girth (7.80 cm), individual fruit weight (107.80 g) and total fruit weight (6.60 kg/ tree) (Sowmiya et al., 2018).

In the cashew ecosystem, four colonies of *A. cerana indica* and *T. iridipennis* per acre increased nut size (length – 3.37 cm thickness – 1.74 cm). The next best results were obtained with two colonies per acre, while the control recorded the lowest thickness (1.58 cm) (Deepika et al., 2021). Similarly, the fruit weight of moringa increased by 9.42% by using honey bees in pollination whereas the length and girth of the fruit showed a 20.34% and 1.10% increase in size (Sowmiya et al., 2018).

The yield parameters of Aonla indicate that the use of honey bee colonies in orchards accounted for an approximately 10.00 – 37.00% increase in the yield of Aonla fruits. Deepika et al. (2021) reported nut yield increases of

17.74% and 19.35% with four colonies of *A. cerana indica* and *T. iridipennis* per acre, while two colonies per acre resulted in yield gains of 6.45% and 8.87% over the control. In mangoes, the highest yield (84.75 q/ha) was recorded in seven *A. cerana indica* hives/ha representing 23.66% increment in yield over the control, followed by five colonies/ha (14.35%) (Deuri et al., 2018). The amount of seeds/fruit in bee-pollinated plots of kiwi fruit ( $24.10 \pm 0.91$ ) was higher than the artificially pollinated flowers ( $1.79 \pm 0.91$  seeds/fruit) (Sáez et al., 2019). According to Chauhan & Singh (2021) an increase of number of seeds/fruit (328.94) and weight of 1000 seeds (71.31%) were observed in watermelon plot pollinated by stingless bees, *T. iridipennis* over pollinator exclusion treatment.

It is concluded that hymenopterans were the abundant insect visitors of the Aonla ecosystem contributing to greater pollination. On observing treatments with different numbers of colonies of honey bees, four colonies of *Tetragonula iridipennis*/acre and four colonies of *Apis cerana indica*/acre accounted for an increase in the qualitative and quantitative parameters of Aonla fruit set and yield.

## Conclusion

According to the results, the stable diversity of insect floral visitors across both locations and seasons contributes to a consistent pollinator community. This suggests that bees and other pollinators can be effectively integrated into the agroecosystems of the study location to regulate pollination and increase productivity. A pollinator-crop combination that performs well in a particular area or covered environment might not function similarly in another. Therefore, site-specific studies are required for specific locations. Future studies should focus on the influence of honey bee density and the effective size of visitation rates on crop productivity.

## Authors' Contribution

VD: Investigation, data curation, formal analysis, software, validation, visualization, writing-original draft, writing-review & editing.

KS: Resources, supervision, project administration, visualization. BU: Conceptualization, methodology, supervision, project administration, visualization.

BS: Formal analysis, validation, visualization, writing-review & editing.

AA: Visualization, writing-review & editing.

RP: Validation, visualization, writing-review & editing.

KB: Formal analysis, validation, visualization.

## Supplementary Material

Supplementary material is available for download at the article webpage.

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