Nectar reoccurrence may increase visitation rates of *Tetragonula iridipennis* , in crop *Cucumis sativus*

Abstract

Since nectar is the main reward, it is essential for drawing in insect pollinators. Pollinator visitation patterns and more general plant-pollinator interactions can be greatly impacted by the dynamic nature of nectar secretion. Although it is well known that nectar is the primary pollinator attractant, little is known about whether nectar replenishes after being removed. To comprehend how nectar availability affects pollinator behavior and the long-term persistence of floral resources, it is imperative to look into the replenishment process. Our findings highlight the dynamic nature of nectar availability suggest that nectar is not entirely depleted in a single pollinator visit, with measurable amounts remaining or replenished over time. These patterns underscore the adaptive significance of nectar recurrence, supporting sustained pollinator activity and enhancing floral resource utility.

Keywords

Nectar , cucumber, stingless bee, pollinator

Introduction

Insect pollination is an ecosystem service that preserves ecosystem services and biodiversity on Earth. It is also essential to the security of human food. Approximately 90% of the angiosperm species that are now known to exist just under 300,000 species in total are pollinated by insects, and over 1500 crops worldwide depend on these services (Christenhusz et.al,2016; Ollerton,2021). Stingless bees are crucial pollinators for numerous plant species, including various cultivated crops (Heard, 1999). The Indian stingless bee, also called the Asian stingless bee or Tetragonula iridipennis (Smith) (Hymenoptera: Apidae), is a member of a sizable monophyletic group of eusocial bees with vestigial or primitive stingers (Chuttong et al., 2016.,Tuksitha et al., 2018; Chauhan and Singh, 2021). Within the genus *Tetragonula*, known for its complexity, there are 34 identified species, with most of them residing in the Indian subcontinent. *Tetragonula iridipennis* (Smith), also referred to as the Indian stingless bee, Dammer bee, or Mosquito bee, is of significant economic importance. This species is prevalent across various Asian countries, including Bangladesh, Bhutan, India, Indonesia, Myanmar, Nepal, Pakistan, and Sri Lanka (Rasmussen, 2013; Rahman et al., 2018; Engel et al., 2019;

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In order for flowering plants to successfully reproduce, nectar is essential for drawing in insect pollinators. Pollinator visitation patterns and general plant-pollinator interactions can be greatly impacted by the dynamic nature of nectar secretion. Although it is commonly known that nectar is the main reward for pollinators, it is still important to research whether nectar replenishes after it is first removed. Comprehending this phenomenon is essential for evaluating pollinator behavior and plant reproductive strategies.

Our goal in this study was to find out how nectar is restored after it has been initially depleted. This study sheds light on the ecological and evolutionary ramifications of nectar dynamics in plant-pollinator systems by investigating whether nectar secretion happens frequently or only once.

Material and methods

Experimental Area

The Vegetable Research Center, GBPUAT, Pantnagar, U.S. Nagar, Uttarakhand, India, was the site of the study. Its geographic coordinates are 29° 1′22.4″ N and 79° 29′52.2″ E, and its elevation is 243.84 meters above mean sea level. The region has a humid subtropical climate, with summer temperatures (May–June) reaching 32°–43° degrees Celsius and winter temperatures (January) ranging from 20° to 10° degrees Celsius.

The crop *Cucumis sativus* was sown in greenhouse at the month of May and flowering start in the mid June. 1 SS HIVE stingless Bee box from meliponiculture site to greenhouse was transferred before the beginning of flowering.

Fig 1-Cucumis sativus crop in Greenhouse

Fi 2-Graduated 5 μL capillary tube inserted to nectary of flower.

Nectar reoccurrence pattern

The amount and concentration of the floral nectar were measured by bagging 10 flower buds from ten plants using voile cotton bags the day before anthesis. Sampling started at 09:00 a.m., when all the flowers were open and bees were starting to visit them. It continued all day, at 2-hour intervals, and from 10 flowers buds from ten plants at each time period, until 17:00 p.m., since most open flowers had abscised from plants by then.
In order to measure the nectar volume, capillary tube of 5 μL were carefully positioned at the base of the flower staminoids so that capillary action would cause the nectar to flow up in the tube (Hocking 1953). The nectar collected was not discarded but utilized in further experiment.

Result and discussion

(Tej *et al*. 2017; Sen *et al.* 2023) have demonstrated the positive impact of stingless bees, particularly *Tetragonula iridipennis*, on the pollination of greenhouse cucumbers in southern India. In our findings also we have observed the positive impact of stingless bees on cucumber crop.

The bar graph represents the different plants and in it the nectar at different time interval

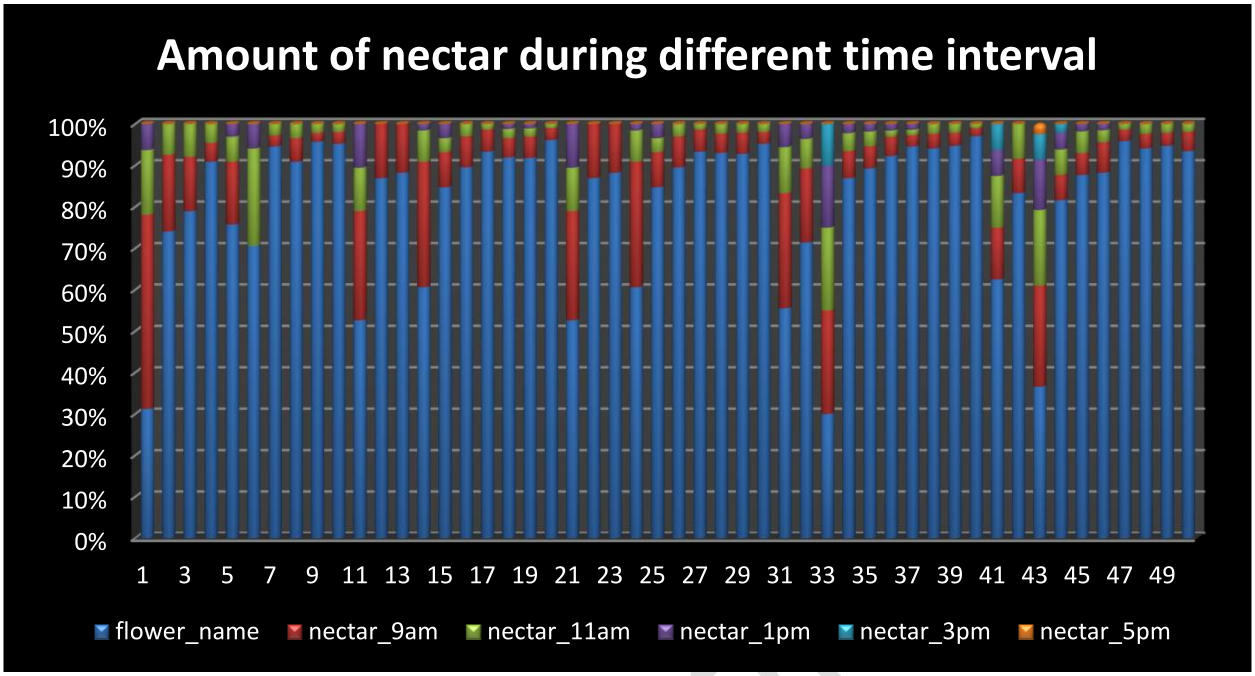

Fig 3- Graphical representation of nectar amount during different time interval

Statistical analyses were used to validate this observation and show that nectar production is a recurrent phenomenon. Nectar that is initially produced may persist and be discovered in subsequent collections. We took nectar production measurements at five different times to account for this. Nectar may continue to be replenished over time, as evidenced by the fact that in certain instances, residual nectar was seen even during the last collection.

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.
nec_t	Pillai's Trace	.692	20.769[b]	4.000	37.000	.000
	Wilks' Lambda	.308	20.769[b]	4.000	37.000	.000
	Hotelling's Trace	2.245	20.769[b]	4.000	37.000	.000
	Roy's Largest Root	2.245	20.769[b]	4.000	37.000	.000
nec_t * flower_name	Pillai's Trace	1.041	1.564	36.000	160.000	.033
	Wilks' Lambda	.286	1.549	36.000	140.393	.038
	Hotelling's Trace	1.541	1.520	36.000	142.000	.045
	Roy's Largest Root	.735	3.266[c]	9.000	40.000	.005

a. Design: Intercept + flower_name

Within Subjects Design: nec_t

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Table 1- multivariate test table

Nectar levels were examined in relation to flower type (flower_name) and time (nec_t) using a Multivariate Analysis of Variance (MANOVA). The results on Table 1 of the analysis showed that time had a substantial main influence and that flower type and time interacted.

Time's Multivariate Effects (nec_t):Time has a substantial impact on nectar levels, according to MANOVA data. Nectar levels vary significantly across time points, according to all four test statistics (Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root) (Pillai's Trace = 0.692, F = 20.769, $p < 0.001$; Wilks' Lambda = 0.308, F = 20.769, $p < 0.001$; Hotelling's Trace = 2.245, F = 20.769, $p < 0.001$; Roy's Largest Root = 2.245, F = 20.769, $p < 0.001$). This implies that time has a major impact on nectar levels.

Interaction Effect of Flower Type and Time (nec_t * flower_name): Time and flower type were found to interact significantly. Nectar levels vary over time among flower types, according to all multivariate tests (Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root) (Pillai's Trace = 1.041, F = 1.564, p = 0.033; Wilks' Lambda = 0.286, F = 1.549, p = 0.038; Hotelling's Trace = 1.541, F = 1.520, p = 0.045; Roy's Largest Root = 0.735, F = 3.266, p = 0.005).

Assumption of Sphericity: The assumption of sphericity was not met, as evidenced by the violation of Mauchly's Test of Sphericity (W = 0.015, $\chi^2 = 162.237$, $p < 0.001$). As a result, the Huynh-Feldt and Greenhouse-Geisser corrections were used in the following studies.

Within-Subjects Effects: substantial results in tests assuming sphericity (F = 20.769, $p < 0.001$) and after applying the Greenhouse-Geisser correction (F = 1.564, p = 0.033) showed that time (nec_t) had a substantial effect on nectar levels across all time points. In certain samples, the relationship between flower type and time was also significant, indicating that nectar levels change over time in distinct ways for each kind of flower.

Effects Between Subjects: Flower type may affect nectar levels, but not sufficiently to reach the traditional threshold for statistical significance ($p < 0.05$), according to the main effect of flower type (flower_name), which was marginally significant (p = 0.063).

Table 2-Tests of Between-Subjects Effects

Measure: MEASURE_1

Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	9.683	1	9.683	39.405	.000
flower_name	4.461	9	.496	2.017	.063
Error	9.829	40	.246		

Table 2: Between-Subjects Effects Analysis for Nectar Recurrence

To determine how different flower (flower_name) affected the dependent variable Average, a between-subjects effects study was conducted. An overall strong effect on the dependent variable was shown by the results, which showed that the intercept was extremely significant ($F = 39.405$, $p < 0.001$). Though the difference did not reach the traditional significance level of $p < 0.05$, the effect of flower_name was marginally non-significant ($F = 2.017$, $p = 0.063$), suggesting a trend towards distinctions between flower types. With 40 degrees of freedom, the error term linked to the between-subjects effect had a mean square of 0.246 and was employed in the F-test for flower_name. All things considered, even though the intercept demonstrated a substantial effect, flower_name's influence on the dependent variable fell short of statistical significance in this analysis.

Significant and complex findings on the variables affecting nectar levels are revealed by the combined study of the Multivariate study of Variance (MANOVA), Mauchly's Test of Sphericity, and Tests of Between-Subjects Effects results. All multivariate test statistics confirmed the significant main impact of time ({nec_t`) on nectar levels, as shown by the MANOVA results (e.g., Pillai’s Trace = 0.692, $F = 20.769$, $p < 0.001$). Furthermore, a noteworthy interaction effect between flower type and time was noted (`nec_t * flower_name`), suggesting that different flower kinds had different temporal variations in nectar levels.

To enable a valid interpretation of the within-subjects effects, Greenhouse-Geisser and Huynh-Feldt corrections had to be applied after Mauchly's Test showed that the within-subjects factor violated the sphericity assumption. The substantial effect of time and the combination with flower type on nectar levels were validated by these adjustments.

Additionally, a highly significant intercept ($F = 39.405$, $p < 0.001$) was revealed by the between-subjects effects analysis, suggesting a large overall effect on nectar levels. A possible but inconclusive influence of flower type was suggested by the marginally non-significant main effect of flower type (flower_name) ($F = 2.017$, $p = 0.063$).

In conclusion, temporal variation is the main factor influencing nectar levels, with notable variations across time points and interactions between flower type and time. Although flower type by alone could not demonstrate statistical significance, its interplay with time highlights how it influences patterns of nectar availability.

The tests show that nectar recurrence is noticeable and cannot be disregarded. The results imply that a flower's nectar is not completely consumed in a single pollinator visit. Rather, a quantifiable quantity of nectar is left behind, or it might be refilled during or soon after the visit. This demonstrates a dynamic process that supports frequent pollinator visits by maintaining nectar availability to a certain degree.

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