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Original article (Orijinal araştırma)

Evaluating the role of insect pollinators in the viability of true seeds of shallot in tropical agroecosystems[1]

Tropikal tarım ekosistemlerinde arpacık soğanı gerçek tohumlarının canlılığında böcek tozlayıcılarının rolünün değerlendirilmesi

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Abstract

Pollen transfer in tropical agroecosystems to increase seed production has received limited research, notably on shallot pollination and insect pollinators. This study evaluated how insect pollinators optimize shallot seed production in tropical agroecosystems, i.e. Batu and Malang Districts in Indonesia. This study was conducted from June to October in 2023. We examined pollinator diversity, foraging behavior, and the effectiveness of dominating insect pollinators during umbel flower anthesis, as well as visiting insect foraging and visiting patterns. We assessed pollinator effectiveness by comparing visitation rates under four treatments. A total of 21 insect species belonging to three orders visited the shallot flowers, of which 14 species have the potential to act as pollinators. *Apis cerana* Fabricius, 1793 (Hymenoptera: Apidae) and *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) emerged as potentially effective pollinators. Air temperature significantly influenced pollinator activity in visiting anthesis umbels. Evaluations of pollinator efficiency showed that *A. cerana* was more efficient than *L. sericata* in promoting the production of true shallot seed and has a significant role in ensuring high-quality pollination. This highlights the necessity of comprehending the specialized contributions of pollinators for shallot seed production.

Keywords: *Allium cepa* var. *aggregatum*, cross pollination, insect pollinator, tropical agroecosystem, true shallot seed

Öz

Polen transferi, tropik tarımsal ekosistemlerde tohum üretimini artırmak için özellikle arpacık soğanı tozlaşması ve böcek tozlaştırıcılar üzerine sınırlı araştırmalara konu olmuştur. Bu çalışma, böcek tozlaştırıcıların Endonezya'nın Batu ve Malang Bölgelerinde tropik tarımsal ekosistemlerde arpacık soğanı tohum üretimini nasıl optimize ettiğini değerlendirmiştir. Bu çalışma Haziran-Ekim 2023 tarihleri arasında gerçekleştirilmiştir. Tozlaştırıcı çeşitliliğini, beslenme davranışlarını ve çiçeklenme dönemi boyunca baskın böcek tozlaştırıcıların etkinliğini, ayrıca ziyaret eden böceklerin beslenme ve ziyaret desenlerini incelenmiştir. Ziyaret oranlarını dört farklı uygulamada karşılaştırarak tozlaştırıcı etkinliğini değerlendirilmiştir. Arpacık soğanı çiçeklerini ziyaret eden, üç gruba ait toplamda 21 böcek türü tespit edildi ve bu türlerin 14'ünün potansiyel tozlaştırıcı olabileceği belirlenmiştir. *Apis cerana* Fabricius, 1793 (Hymenoptera: Apidae) ve *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) potansiyel olarak etkili tozlaştırıcılar olarak ortaya çıkmıştır. Hava sıcaklığı, çiçeklenme döneminde tozlaştırıcı aktivitesini önemli ölçüde etkiledi. Tozlaştırıcı verimliliğinin değerlendirilmesi, *A. cerana*'nın gerçek arpacık soğanı tohumu üretimini teşvik etmede *L. sericata*'dan daha verimli olduğunu ve yüksek kaliteli tozlaşmayı sağlamakta önemli bir rol oynadığını göstermiştir. Bu durum, arpacık soğanı tohum üretimi için tozlaştırıcıların özel sorumluluklarını anlamanın gerekliliğini vurgulamaktadır.

Anahtar sözcükler: *Allium cepa* var. *agregatum*, çapraz tozlaşma, böcek tozlaştırıcı, tropikal tarımsal ekosistem, arpacık tohumu

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Introduction

Shallot, *Allium cepa*. var. *aggregatum* G.Don (Asparagales: Amaryllicidaceae) is a globally significant agricultural crop due to its substantial contribution to food security and socioeconomic development, especially in tropical areas. Originally native to subtropical regions, shallots are extensively cultivated, including in tropical areas. Shallot seed production as known True Shallot Seeds (TSS), is a critical aspect of shallot cultivation for bulb formation. TSS production technology plays a pivotal role in the plant's life cycle, as shallot plants rely on cross-pollination to produce high-quality seeds. Utilizing TSS as seeds offers various benefits, such as increased productivity, reduced production costs, and enhanced market acceptance, making it appealing to farmers (Askari-Khorasgani & Pessarakli, 2019). Insect pollinators are critical in the TSS production process because they facilitate pollen transfer from male to female flowers, ensuring the formation of quality seeds.

In tropical agroecosystems, research on the effectiveness of insect pollinators of shallot in TSS formation is still limited, unlike in subtropical agroecosystems, where much more research has been conducted (Monasterio et al., 2023). Tropical agroecosystems tend to have more extreme climatic characteristics, with higher temperatures and greater humidity throughout the year, often experiencing more unpredictable rainfall patterns and higher intensity. These climatic factors can affect pollinator activity and TSS formation. On the other hand, subtropical agroecosystems tend to have more regular seasonal variations, with more stable temperatures and rainfall. The differences in environmental conditions between tropical and subtropical regions can directly impact pollinator diversity and behavior, as well as their effectiveness in seed formation.

Tropical regions such as Indonesia cultivate shallots using monoculture systems and extensive insecticide applications (Nurjati et al., 2018). Monoculture practices result in reduced plant diversity, altering habitats, and affecting pollinator populations (Tarakini et al., 2021; Bukhari et al., 2024). Intensive insecticide use disrupts insect community, diminishing the essential pollinator presence for effective pollination (Kumari & Rana, 2018; Buszewski et al., 2019). Consequently, the reliance on insect pollinators directly impacts the efficacy of true shallot seed formation. These environmental shifts highlight the necessity for a deeper comprehension of the interplay between prevalent agricultural practices in tropical agroecosystems and the dynamics of pollinator populations, including their behaviors, and their consequent effects on true shallot seed production. This understanding is critical for supporting Indonesian shallot cultivation's sustainability.

Previous research has highlighted the crucial role of specific insect species in shallot pollination. Palupi et al. (2015) demonstrated that *Apis cerana* Fabricius, 1793, *Trigona* sp. Jurine, 1807 (Hymenoptera: Apidae), and *Lucilia* sp. Robineau-Desvoidy, 1830 (Diptera: Calliphoridae) significantly contribute to shallot pollination, enhancing both the production and quality of true shallot seeds (TSS). In subtropical regions, various bee species [*A. cerana*, *Apis dorsata* Fabricius, 1793, and *Apis florea* Fabricius, 1787 (Hymenoptera: Apidae)] and fly species [*Episyrphus balteatus* (De Geer, 1776), *Eristalinus aeneus* (Scopoli, 1763), (Diptera: Syrphidae) and *Calliphora vicina* Robineau-Desvoidy, 1830) (Diptera: Calliphoridae)] have been identified as pollinators for *Allium* spp. (Abrol, 2006; Devi et al., 2015). Pangestuti et al. (2023) confirmed the significance of *A. cerana*, *Trigona* sp., and *Lucilia* sp. in shallot pollination in tropical regions. However, the specific contributions of each pollinator to TSS quality remain understudied.

In tropical regions, the Calliphoridae family (Diptera) plays a notable role in shallot flower pollination. Understanding the diversity and foraging behavior of these pollinators provides valuable insights into tropical ecosystem biodiversity and has significant implications for agricultural productivity in tropical agroecosystems. Investigating the composition and foraging behavior of flower-visiting insects, particularly potential shallot pollinators, is crucial for developing effective pollinator conservation strategies and improving crop yields. Therefore, conducting comprehensive studies on flower-visiting insect diversity is essential for strengthening the foundation of pollinator conservation and enhancing agricultural productivity in tropical agroecosystems.

This study aims to comprehensively examine the role of insect pollinators in shallot pollination success and their impact on TSS production and quality in tropical regions. The research seeks to assess pollinator diversity through various indicators such as diversity indices, evenness, and dominance of both visitor and pollinator insects on shallot umbels. It also analyzes the foraging behavior of key insect pollinators by examining their visitation patterns during shallot flower anthesis. Furthermore, the study evaluates the effectiveness of different pollinator species in facilitating successful pollination of shallot umbels and investigates the relationship between pollinator activity and the quantity and quality of TSS produced. By adopting this multifaceted approach, the research endeavors to advance scientific understanding of the intricate interaction between insect pollinators and shallots in tropical agroecosystems. Additionally, this study aims to provide crucial insights for developing targeted pollinator conservation strategies and enhancing shallot agricultural productivity, with a specific focus on improving TSS production and quality in tropical regions.

Materials and Methods

This study comprised several components: documenting all insects visiting shallot flowers during anthesis, analyzing the pollinators, and evaluating the effectiveness of these pollinators in enhancing the production of high-quality TSS.

Diversity and composition of insect species visiting shallot flowers

The inventory of insects visiting shallots and identification of potential insect pollinators during flower anthesis were conducted at two different altitudes, namely Batu and Ngantang, Indonesia, from June to September 2023. Both observation locations were in the shallot cultivation centers in Batu and Ngantang. The agroecosystems in both observation locations were similar, consisting of monoculture shallot plants of the “Tajuk” variety, with intensified pesticide spraying every 2-5 days when the plants were 1-5 weeks after planting (WAP). The observation plots, measuring 4 m by 1 m, contained 2100 plants. The observation plots received no pesticide spraying (Table 1).

Table 1. Geographical description of research locations for the inventory of insects visiting shallot flowers

Location	Coordinate	Altitude (m asl)	Altitude
Batu, Batu City	7°52'31"S,112°31'20"E	848.5	High
Ngantang, Malang	7°52'49"S,112°34'22"E	625.7	Moderate

We conducted the inventory of insects visiting the shallot flowers (umbels) during flower anthesis, when the plants were 9-10 WAP. The term "flower anthesis" refers to the period when a flower is fully open and functional, often marked by the expansion and blooming of petals or florets arranged in an umbel. We documented the types and numbers of insects visiting the umbels during anthesis. These observations occurred at specific time intervals: (06:00-08:00), (08:00-10:00), (10:00-12:00), (12:00-14:00), (16:00-16:00), and (16:00-17:00), over a period of six consecutive days. These observations revealed that 30-70% of the florets in each umbel had fully opened.

We sampled a total of 10 randomly selected plants representing bloomed umbels. The observation time was 5 minutes per umbel. We collected insects visiting umbels using a sweep net (diameter 30 cm). We pinned and dried the captured insects for further storage and identification. We recorded the air temperature and humidity during each observation. Identification of visiting insects was done by separating them by Order, then grouped into families using insect identification guidelines such as Borror et al. (1954) and Goulet et al. (1993). Identification to the species level was conducted through morphological observation, comparing specimens with the insect collection reference at the Museum Zoologicum Bogoriense, National Research and Innovation Agency in Cibinong, Bogor. For pollinator analysis, all captured visiting insects underwent microscopic examination to detect the presence of pollen grains on their bodies.

Assessment of the effectiveness of shallot pollinators

To assess effective pollinators, we conducted a pollinator analysis at the Laboratory of Entomological Research Group in Malang, Indonesia, from August to October 2023. This analysis involved microscopic examination of insect visitors to identify potential pollinators based on the presence of pollen grains on their bodies. We focused on pollen found on body parts other than specialized pollen-carrying structures like pollen baskets. This approach allowed us to distinguish between insects that might incidentally carry pollen and those more likely to contribute to pollination. Our analysis identified two species, *Apis cerana* and *Lucilia sericata* (Meigen, 1826), as potential pollinators due to the presence of pollen grains on their bodies.

We calculated the diversity and composition of insect species visiting shallot flowers using the Shannon-Wiener diversity indices, which include the evenness index, species richness index, and dominance index (Odum, 1971).

The research locations were conducted at shallot planting centers of the “Tajuk variety in Batu (7°52'56", 112°32'35"). We selected shallot plants in 3 plots (4 m x 1 m) for this assessment, ensuring each plant had two umbels. Ten plants were observed, for a total of 20 umbels per treatment. We specifically selected two pollinator species for this study: *A. cerana* and *L. sericata*, which were determined to be the most often visited pollinators based on our previous analysis. The objective of this experiment was to assess the efficacy of pollinators by introducing several pollinator species to enclosed shallot plants, with the assumption that only the chosen pollinator species would facilitate pollination. The cage's dimensions were 50 cm x 50 cm x 100 cm, and it was equipped with a 50 mesh screen. The treatments applied were:

1. Shallot plants were caged; 2 individuals *A. cerana* were introduced into the cage for 6 days when 70% of the florets per umbel had bloomed (Caged + *A. cerana* - CAc).
2. Shallot plants were caged; 2 individuals were *L. sericata* introduced into the cage for 6 days when 70% of the florets per umbel had bloomed (Caged + *L. sericata* - CLs.).
3. Shallot plants in open condition/without cage (Control-opened- CO).
4. Shallot plants were caged without pollinator introduction (Control-closed-CC).

For the caged treatments and pollinator release experiment, we used specially designed cages that enclosed individual shallot plants. Each cage contained two umbels undergoing anthesis to ensure sufficient floral resources. We introduced two individual pollinators of the same species (either *A.cerana* or *L. sericata*) into each cage at the beginning of each day's observation period.

The pollinator release was conducted daily from 7 am to 2 pm, coinciding with the peak foraging activity of these species. This process was repeated for six consecutive days to cover the full anthesis period of the shallot umbels. Importantly, we used new, naive insects each day to prevent habituation or exhaustion. These fresh pollinators had not previously visited shallot flowers, ensuring unbiased foraging behavior. At the end of each day's 6-hour observation period, the insects were removed from the cages. This approach allowed us to control exposure time precisely while maintaining the insects' well-being. The use of fresh pollinators each day eliminated concerns about long-term confinement effects on insect behavior or survival.

Observations were made on various parameters, including the number of florets per umbel, the number of capsules per umbel, the number of TSS per umbel, the weight of 100 seeds, and TSS viability as qualified seeds. Seed viability tests were conducted using 100 seeds per treatment with six replicates. Observations of TSS viability included TSS germination rate, and the proportion of germinated seeds that grew normally and abnormally.

Data analysis

The diversity of visiting insects and potential pollinators was analyzed using Shannon's formula, diversity indices, evenness index, species richness index, and dominance index (Odum, 1971). To evaluate the relationship between temperature and relative air humidity and the number of pollinators visited to umbels during anthesis, linear regression analysis was done for each environmental condition factor. The effectiveness test of pollinator performance was conducted using analysis of variance (ANOVA), followed by Fisher’s PLSD test for each observation parameter. We used linear regression analysis to look at the link between seed weight, germination capacity, or seed germination rate, and the percentage of germinated seeds that grew normally for the TSS viability test as qualified seeds. All statistical analyses were performed using Minitab 19.2 Statistical Software (Minitab, LLC, 2020).

Results and Discussion

Results

Diversity and composition of insect species visiting shallot flowers

This study examined the variety and composition of insect species visiting shallot umbels during anthesis. A total of 21 insect species from 11 families and three orders (Hymenoptera, Diptera, and Lepidoptera) were identified at two different elevations (Table 2 and 3). The Hymenoptera order, particularly the Apidae family, exhibited the highest species diversity and the greatest number of individual visitors at both locations. Among the 21 species, 14 were classified as potential pollinators, while 7 were categorized as mere visitors (Table 3).

Table 2. Number of species and individual of Pollinators on Shallot Umbel During Anthesis at High Altitude (Batu) and Moderate Altitude (Ngantang) Agroecosystems

No	Order	Family	Number of Species	Number of individuals at	
				Batu	Ngantang
1	Diptera	Calliphoridae	2	124	120
2		Phoridae	2	5	1
3		Syrphidae	1	6	51
4	Hymenoptera	Apidae	6	151	78
5		Halictidae	2	7	3
6		Chalcididea	1	2	0
7		Formicidae	1	4	2
8		Vespidae	2	2	11
9	Lepidoptera	Erebridae	1	2	1
10		Noctuidae	1	24	6
11		Nymphalidae	1	2	0

Table 3 shows that the species of insects that visited shallot flowers during anthesis were different in the agroecosystems at high altitude (Batu) and mid-altitude (Ngantang). The diversity index for the family of visitors and species of pollinators in the agroecosystem at high altitude (Batu) and mid-altitude (Ngantang) was moderate. However, the diversity index for the family of pollinators was low in both agroecosystems. This indicates that in both agroecosystems, the number of pollinator insect families is low, or their diversity is limited (Table 4).

The composition of pollinators observed visiting shallot flowers during the anthesis period revealed distinctive patterns across different agroecosystems. *Lucilia sericata* appeared to be a prevalent visitor to both medium (Ngantang) and highland (Batu) agroecosystems, exhibiting high frequency across both environments (Figure 1). Following this trend, *A. cerana* also demonstrated notable activity in these agroecosystems. Conversely, *T. iridipennis* exhibited a preference for visiting shallot agroecosystems in highland regions, while *C. megacephala* and *E. balteatus* displayed a greater inclination towards shallot agroecosystems situated in medium agroecosystems. Interestingly, pollinators from the Apidae family consistently exhibited intensive visitation patterns across both types of agroecosystems. This description of how pollinators behave helps us understand the complex dynamics that affect pollination interactions in shallot agroecosystems in a range of environmental conditions. Based on these observations, we can conclude that *L. sericata* and the three Apidae species are frequent visitors to shallot umbels and have significant potential as effective pollinators.

Table 3. List of visitor insects and potential pollinators in shallot flowers during anthesis at high altitude (Batu) and moderate altitude (Ngantang) agroecosystems

No	Species	Ordo:Family	V/P	Number of individuals at	
				Batu	Ngantang
1	*Apis cerana* F.	Hymenoptera: Apidae	P	34	53
2	*Apis florea* F.	Hymenoptera: Apidae	P	57	11
3	*Tetragonula iridipennis* (Smith)	Hymenoptera: Apidae	P	37	21
4	*Trigonula* sp.	Hymenoptera: Apidae	P	0	0
5	*Amegila zonata*	Hymenoptera: Apidae	P	6	6
6	*Brachymeria lasus* (Walker)	Hymenoptera: Chalcididae	V	2	0
7	*Polyrachis* sp	Hymenoptera: Formicidae	V	4	2
8	*Nomia* sp1	Hymenoptera: Halticidae	P	4	2
9	*Nomia* sp2	Hymenoptera: Halticidae	P	3	1
10	*Sceliphon javanum* (Lepeletier de S. F)	Hymenoptera: Sphecidae	V	1	1
11	*Cerceris* sp.	Hymenoptera: Sphecidae	V	1	1
12	*Euodynerus* sp	Hymenoptera: Vespidae	V	1	1
13	*Delta campaniforme* (F.)	Hymenoptera: Vespidae	V	2	1
14	*Lucilia sericata* (Meigen)	Diptera: Calliphoridae	P	124	120
15	*Chrysomya megacephala* (F.)	Diptera: Calliphoridae	P	20	15
16	*Episyrphus balteatus*	Diptera: Syrphidae	P	6	51
17	*Graptomyza* sp.	Diptera: Syrphidae	P	1	0
18	*Musca domestica* L.	Diptera: Muscidae	V	16	10
19	*Amata huebneri* (Boisduval)	Lepidoptera: Erebidae	V	2	2
20	*Spodoptera exigua* Hübner	Lepidoptera: Noctuidae	V	24	6
21	*Ariadne ariadne* (L.)	Lepidoptera: Nymphalid	V	2	2

Note: P: Insects that visit and potentially act as pollinators; V: Insects that only visit and did not potentially act as pollinators based on pollinator analysis.

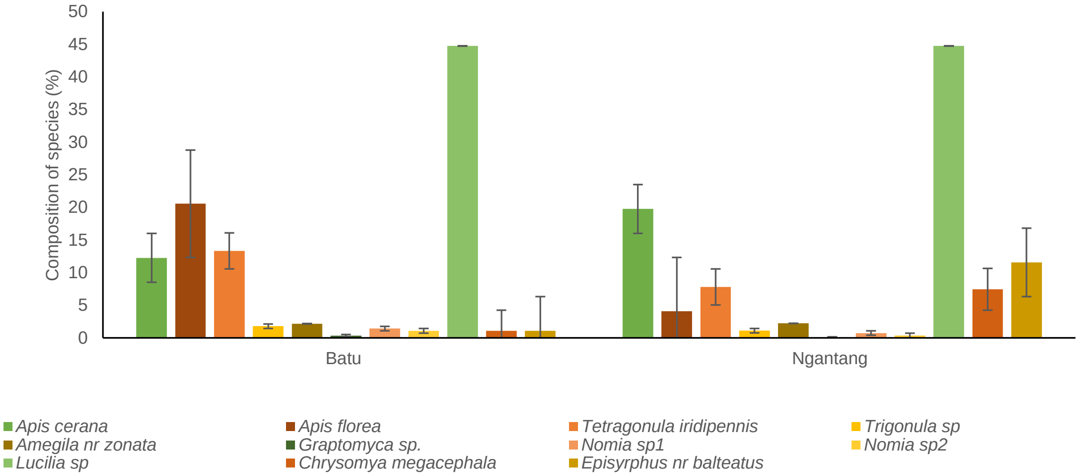

Figure 1. Composition (Means ± Standard error) of shallot pollinator species in shallot agroecosystems at high- (Batu) and moderate- (Ngantang) altitudes.

The values of the evenness index for the visitor and pollinator families, and species of pollinators were all less than 0.4, indicating low evenness in the distribution of visitor and pollinator families and species based on the Shannon-Wiener evenness index category (Table 4). This suggested a relatively uniform distribution of pollinators and visitors within the agroecosystem. The richness indices for the family of all visitors and species of pollinators in both agroecosystems showed higher values compared to the family of pollinators. This indicates that while there were fewer pollinator families compared to visitor families, there was a relatively high number of pollinator species. However, the richness index values for all three parameters were relatively low in both highland and midland agroecosystems. In both midland and highland agroecosystems, the dominance index for all three parameters was low, indicating no dominant species among visitors or pollinators in the shallot agroecosystem.

Table 4. Diversity of visiting insects during shallot flower anthesis in agroecosystems at high altitude, B (Batu) and moderate altitude, N (Ngantang) based on Shannon-Wiener indices

Parameters	Diversity index (H')		Evenness index (E)		Richness Index (R)		Dominancy Index (C)	
	B	N	B	N	B	N	B	N
Family of Visitors	1.312	1.373	0.119	0.125	1.725	1.783	0.359	0.312
Family of Pollinators	0.780	0.452	0.130	0.041	0.176	0.185	0.452	0.278
Species of Pollinator	1.000	1.556	0.100	0.141	1.762	1.805	0.271	0.290

We observed a clear pattern of high pollinator activity at regular times, especially from 6:00 a.m. to 12:00 p.m., in the tropical shallot agroecosystems in the highlands (800 m) and midlands (600 m). Specifically, at the highland site, the number of Dipteran pollinators exceeds that of Hymenopteran pollinators between 12:00 and 17:00 (Figures 2A and 2B). The pollinating insects that visit shallot umbels on a regular basis come from three Hymenopteran families and two Dipteran families. Among the Hymenopteran pollinators, individuals from the Apidae family and Dipteran pollinators from the Calliphoridae family dominated in abundance compared to other families (Figure 2C). The Apidae consisted of three species, namely *A. cerana, A. florea*, and *T. iridipennis*, and two species, *L. sericata* and *C. megachepala*, comprised the Calliphoridae family (Table 3).

The regression analysis revealed that temperature significantly influenced *L. sericata* and *C. megacephala*, contributing 38% and 31%, respectively, at both agroecosystem sites, while humidity did not affect their activity at the midland site. However, it is linearly related, contributing only 11% to the highland agroecosystem.

Assessment of the effectiveness of shallot pollinators

A quantitative assessment of successful pollination, based on the number of fruit capsules formed, demonstrated the effective transfer of pollen grains to the stigmas of shallot flowers. When comparing treatments, the CO and CAc treatments significantly exhibited higher rates of fruit capsule formation than the CC and CLc treatments (Table 5). This suggests that the presence of *A. cerana* in the CAc treatment closely replicates natural pollination processes. Furthermore, the proportion of seed formation reinforced the effectiveness of pollination. Among all treatments, the OC treatment produced the most seeds, followed by the CAc treatment with *A. cerana*, the CLs treatment with *L. sericata*, and the CC treatment. These results highlight the importance of both pollination effectiveness and the treatment environment in determining the reproductive success of shallot plants. In the CC treatment, the percentage of successful pollination was very low and significantly different from other treatments. This indicates that pollination cannot occur optimally in conditions where environmental factors and insect pollinators do not have access to the flowers.

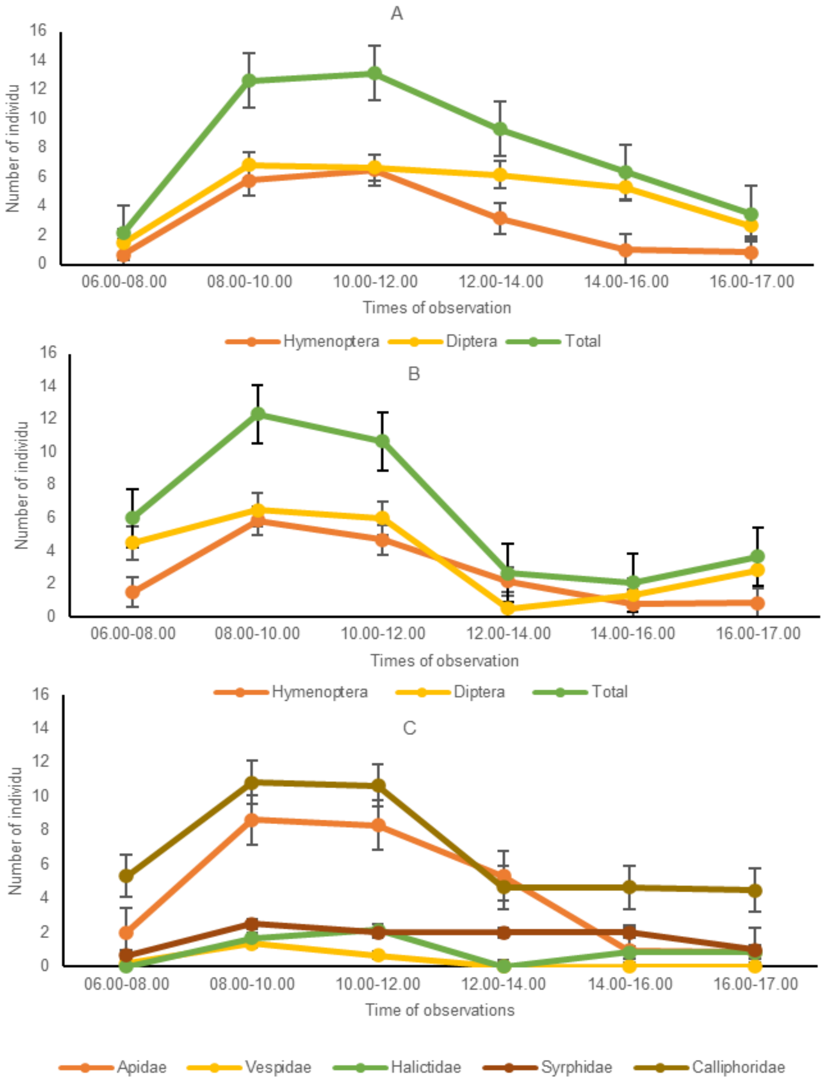

Figure 2. Diel pattern of shallot flower pollinator activity in Batu and Ngantang (Mean number of individuals ± Standard Error) during umbel anthesis. A. Diel pattern of pollinators in Ngantang; B. Diel pattern of pollinators in Batu; C. Diel pattern of Hymenopteran and Dipteran families.

Table 5. Means (± standard error) of number of florets per umbel, number and percentage of fruit capsules' formation per umbel, number of seeds formed per umbel, and percentage of successful pollination in the treatments: non-caged (Open control), caged (Closed control), caged with the introduction of *A. cerana*, and caged with the introduction of *L. sericata*. in shallot plants

Treatment	Number of florets/umbels	Number of fruit capsules/umbel	% Capsule formation[1]	Number of seeds/umbels	% successful fertilization[2]
Caged+*A. cerana* (CAc)	83.3 ± 4.0 ab	65.5 ± 3.3 a	80.2 ± 5.3 a	50.6 ± 6.7 a	63.6 ± 7.5 b
Caged+*L. sericata* (CLs)	71.6 ± 5.5 b	28.7 ± 1.9 b	41.9 + 4.2 b	15.8 ± 1.8 b	38.9 ± 4.4 c
Closed Control (CC)	74.7 + 2.8 b	11.5 ± 1.4 c	15.6 + 2.0 c	0.7 ± 0.3 c	5.6 ± 2.5 d
Open control (OC)	90.5 ± 7.1 a	62.9 ± 5.7 a	70.2 ± 3.8 a	57.1 ± 4.1 a	82.7 ± 5.5 a

[1] The percentage of capsule formation indicates the plant's ability to successfully generate fruit following fertilization.

[2] The percentage of successful fertilization is an indicator of the successful conversion of fertilization into the development of fruit and seeds.

Different letters on values corresponding to each response variable of treatments indicated significant differences between the treatments according to Fisher's PLSD test ($p < 0.05$).

Viability test of the true shallot seeds (TSS)

This test aimed to determine whether pollination from the plots in the pollinator effectiveness test produced TSS suitable for use as high-quality seeds. The weight of TSS obtained from the CAc treatment was not significantly different from the open control (OC) and closed control (CC), with values ranging between 0.29 g and 0.32 g. However, the weight of TSS from the CLs treatment was lower than that of the CAc treatment and even OC, measuring 0.24 g (Table 6). This indicates that pollination in the CLs treatment was less optimal compared to the CA and OC treatments, resulting in the formation of lighter seeds

Table 6. Parameters indicating the viability of True Shallot Seeds (TSS) (Mean ± Standard Deviation) obtained from the effectiveness test on shallot agroecosystems in tropical regions

Parameter	Treatments			
	Caged+*A. cerana*) (CAc)	Caged+*Lucilia sericata*) (CLc)	Opened Control (OC)	Closed Control (CC)
Weight of 100 seeds (g)	0.32 ± 0.04 a	0.24 ± 0.04 b	0.30 ± 0.05 a	0.29 ± 0.02 ab
Seed germination rate (%)	52.29 ± 5.3 a	44.14 ± 10.7 b	35.57 ± 4.5 c	58.71 ± 5.4 a
Normal germination (%)	90.48 ± 3.3 a	59.05 ± 19.05 b	81.45 ± 5.70 a	42.48 ± 4.43 c
Abnormal germination (%)	9.52 ± 3.3 b	40.95 ± 19.05 a	18.55 ± 5.70 b	49.44 ± 5.45 a

Notes: Values in the same rows (parameters) followed by different letters indicate significantly different (p< 0,05) based on Fisher's PLSD tests.

Germination capacity indicates the seed's ability to grow normally and produce normally under optimal environmental conditions.

Abnormal seedling growth refers to seeds that are capable of germination but fail to develop further, such as those without root growth or with irregular stem development.

The germination rate of TSS in the CAc and CC treatments was higher and significantly different from that in the CLs and OC treatments. The caged treatment, with the introduction of *A. cerana* (CAc), had an average total of 50.6 seeds per umbel, indicating successful pollination, whereas the OC treatment had an average of 57.1 seeds per umbel. There was no significant difference between these two treatments. The CC treatment and the caged treatment with *L. sericata*, on the other hand, had lower average TSS values, with 0.7 seeds per umbel for the CC treatment and 14.6 seeds per umbel for the caged treatment (Table 6).

We calculated the percentage of successful pollination by dividing the average TSS per umbel by the total number of fruit capsules per umbel. The pollination success rate in the OC and CC treatments showed no significant difference, whereas the CAc treatment exhibited a lower success rate compared to both controls. Furthermore, the caged treatment, which included *L. sericata*, demonstrated the lowest success rate. The high percentage of successful pollination in CC resulted from the low number of fruit capsules formed per umbel (Table 5). Conversely, in the OC treatment, where pollination occurred with the aid of pollinators and wind, the percentage of successful pollination was optimal, reaching 82.7% (Table 5). In CAc, only 39% of pollinations were successful, which is almost half of the open control. However, this was still a higher and significantly different success rate than in the caged treatment with *L. sericata* (treatment CLs). Therefore, we can conclude that *A. cerana* outperformed *L. sericata* as a superior pollinator in facilitating the formation of TSS.

The linear regression model did not reveal a significant relationship between the rate of germination and the TSS weight (p> 0.05), indicating that the relationship might be non-linear. The TSS weight accounted for 19.68% of normal seed growth (Table 7). In the CC and CLs treatments, TSS weight was lower compared to the open control and CAc treatments (Table 6), resulting in reduced normal germination growth. Although the germination rate in the CC and CLs treatments was not significantly different from that in the CAc treatment and was higher than the OC, it did not lead to optimal growth. Therefore, we concluded that *A. cerana* significantly influences pollination, resulting in the production of high-quality TSS. Conversely, *L. sericata* was less efficient at pollinating shallots and producing TSS of comparable quality. Natural cross-pollination, *i.e.* OC treatment, also produced TSS with an ideal weight and low germination rate (Table 6); however, there was a significantly higher likelihood that this successful germination would result in the emergence of a healthy and prosperous plant.

Table 7. Linear regression analysis between tss weight, germination rate, and normal seed growth

Regression	p value	S	R-sq	R-sq(adj)	R-sq(pred)
TSS weight vs germination rate	0.379	0.05091	2.99%	0.00%	0.00%
TSS weight vs normal seed growth	0.010	0.04546	22.66%	19.68%	10.64%

Note: - S (Standard Error of the Estimate): This is an estimate of the average deviation of the observed values from the values predicted by the model.

- R-squared (R-sq): This is the coefficient of determination, indicating the proportion of variation in the dependent variable that can be explained by the independent variable in the regression model.
- Adjusted R-squared (R-sq(adj)): This indicates the estimated accuracy and quality of the model.
- Predicted R-squared (R-sq(pred)): This provides an indication of how well the model can generalize patterns from the training data to the test data

Discussion

Our study revealed a diverse array of insect visitors to senescing shallot flowers, with the Hymenoptera order, particularly the Apidae family, exhibiting the highest species diversity and visitation frequency across both high and moderate altitude agroecosystems. This aligns with previous research highlighting the importance of Apidae in shallot pollination (Palupi et al., 2015; Davidar & Carr 2015). Among the 14 species classified as potential pollinators, *L. sericata* and three Apidae species (*Apis cerana, Apis florea*, and *Tetragonula iridipennis*) emerged as frequent visitors, suggesting their potential as effective pollinators. The consistent presence of these species across different altitudes indicates their adaptability to various environmental conditions, a characteristic crucial for reliable pollination services in diverse shallot-growing regions (Garibaldi et al., 2019).

The moderate diversity of pollinator species, coupled with low family-level diversity and evenness, suggests a concentration of pollination services among a few key species. This finding highlights the potential vulnerability of shallot pollination to declines in these specific pollinator populations, a concern reverberated in recent studies on crop pollination systems (Reilly et al., 2020). The frequent visits of *L. sericata, A. cerana, T. iridipennis,* and *A. florea* during anthesis likely contribute significantly to pollen transfer and subsequent TSS formation. However, as Mallinger et al. (2021) emphasize, visitation frequency alone does not guarantee pollination efficiency; factors such as pollen load, foraging behavior, and morphological compatibility also play crucial roles in determining a species' effectiveness as a pollinator and its impact on TSS formation and quality.

Several interconnected factors influence the low diversity of visitors and pollinators observed in this research, particularly in heavily sprayed shallot plantations' centers. Heavy insecticide application may directly kill or indirectly affect pollinators by reducing their abundance, diversity, and foraging behavior (Vanbergen et al., 2013; Bloom et al., 2021). Pesticides may also disrupt pollinator foraging behavior by altering floral scent, nectar availability, and flower attractiveness. Furthermore, intensive agricultural practices such as monoculture cropping systems common in large-scale plantations can lead to habitat loss and fragmentation, limiting suitable nesting sites, food resources, and shelter for pollinators. Moreover, monoculture cropping systems often provide a limited range of flowering plants for pollinators, especially during non-crop blooming periods. Thus, the complexity of relationships between farm management, pesticide use, changing habitats, and pollinator movements in tropical agroecosystems contributes to the observed lack of diversity in visitors and pollinators in heavily sprayed shallot plantations.

Balancing agricultural productivity with biodiversity conservation is essential for promoting sustainable farming practices that support pollinator health and ecosystem resilience. Comparative research on insect community diversity indices in these agroecosystems can provide insights into what promotes insect community diversity and how they interact with crops like shallot. The abundance and diversity of Hymenoptera insects, particularly bees, underscore their importance in upholding biodiversity and ecosystem functioning, with extensive research documenting their effectiveness in pollinating a wide range of plant species, particularly *Allium* spp. (Anoosha et al., 2020; Divija & Jayanthi, 2022; Soto et al., 2023).

The study findings suggest that hymenopteran pollinators, particularly members of the Apidae family, especially *A. cerana*, significantly influence pollination dynamics and subsequent TSS production within tropical agroecosystems. Research on the feasibility and perception of TSS technology further reinforces the benefits and practicality of utilizing it for shallot farming (Rahayu et al., 2019). Additionally, TSS offers advantages over traditional seed bulbs, including lower volume requirements, simpler storage and transportation, and healthier plant yields (Hasanah et al., 2022; Marpaung et al., 2023).

Understanding the specific contributions of pollinators to the TSS pollination process is crucial for increasing both productivity and quality. Conservation initiatives should prioritize preserving efficient pollinator species and implementing management strategies that promote their proliferation and activity, reinforcing crop yields and seed quality in onion cultivation. Recent studies have highlighted the effectiveness of floral plantings in bolstering wild bee populations and improving pollination services, particularly for crops reliant on insect pollination, emphasizing the importance of meticulous planning concerning planting dimensions, geographic positioning, and landscape attributes to maximize crop pollination efficacy (Blaauw & Isaacs, 2014).

Solitary bees and other native pollinators are vital components of agricultural ecosystems, conferring significant economic and ecological advantages to agricultural sectors and indigenous habitats. Consequently, activities to mitigate habitat degradation and loss are imperative to preserve environments crucial for sustaining these invaluable pollinator populations (Kline & Joshi, 2020).

Conclusion

Insect pollinators play a vital role in ensuring the viability of true shallot seeds in tropical agroecosystems. Their activity directly influences both the quantity and quality of TSS produced. Among the diverse pollinator community, *Apis cerana* stands out as a particularly effective pollinator, suggesting its potential for managed pollination services. However, the study also highlights the vulnerability of the pollination system to agricultural intensification and the need for conservation efforts to maintain pollinator diversity. These findings have significant implications for shallot cultivation practices and pollinator management strategies in tropical regions, emphasizing the need to integrate pollinator conservation into agricultural systems to ensure sustainable and high-quality TSS production.

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