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Visual and Olfactory Cues with Nectar Accessibility: Drivers of Floral Selection in *Sycanus annulicornis* (Hemiptera: Reduviidae)

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ABSTRACT

Sycanus annulicornis (assassin bug) is a potential predator of agricultural pests in soybean, rice, vegetable, and plantation crops. However, habitat degradation has reduced its population and effectiveness as a biological control agent. This study aimed to evaluate the floral selection of *S. annulicornis* based on visual and olfactory cues, as well as nectar accessibility. Behavior observations were conducted using choice and non-choice tests to assess visitation rates to different flower colors and plant species. The results showed a broad color preference, with the highest visitation rates to white (2.00 ± 0.82) and yellow (1.75 ± 0.63) flowers. In non-choice tests, the highest visitation percentages were recorded on *Tagetes erecta* (82%), *Turnera subulata* (80%), *Turnera ulmifolia* (73%), and *Wedelia trilobata* (67%). In choice tests, *T. subulata* was the most attractive plant, followed by *T. ulmifolia*, *T. erecta*, and *W. trilobata*. Floral morphological compatibility, particularly nectar accessibility, supported the utilization of supplementary food resources by *S. annulicornis*. These findings suggest that selecting appropriate flowering plants can be an effective conservation strategy to enhance the sustainability of *S. annulicornis* populations in agroecosystems.

1. Introduction

Sycanus annulicornis (assassin bug) is a potential predator that acts as a biological control agent for various agricultural commodities, including soybeans, rice, vegetables, and plantation crops such as oil palm (Nasir *et al*. 2023; Zhao *et al*. 2023). Its predatory effectiveness has been demonstrated through field releases that successfully suppressed the population of Setothosea asigna in oil palm plantations (Agus Salim *et al*. 2026). This predator is euryphagous and adaptive, capable of preying on various types of pests such as *Spodoptera litura*, *Plutella xylostella* (Ahmad *et al*. 2016; Rajan *et al*. 2017; Truong *et al*. 2020), *Crocidolomia pavonana* (Sahid & Natawigena 2018), and *Mahasena corbetti* (Nasir *et al*. 2023).

Despite its high potential, the population of *S. annulicornis* in the field tends to decline due to habitat unsuitability (Muhlison *et al*. 2025). This is particularly true in monoculture agricultural systems, which reduce the diversity of food sources and shelter (Grundy & Maelzer 2003; Van Rijn & Wackers 2016). Therefore, conservation strategies for natural enemies through the provision of alternative habitats, such as flowering plants, are important to support their continued function in pest control (Guillermo-Ferreira *et al*. 2012; Gill-Santana *et al*. 2022).

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Flowering plants function as attractants by providing additional food sources in the form of nectar and shelter (Grundy & Maelzer 2003). However, the effectiveness of flowering plants in attracting predators is species-specific (Wackers 2005; Van Rijn & Wackers 2016; Jamian 2017). For example, the minute pirate bug *Orius insidiosus* (Hemiptera: Anthocoridae) is more attracted to flowering strawberry plants than to other flowering plants (Lorenzo *et al*. 2021). In a similar study, *Helianthus annuus* was found to be more attractive to the multicolored Asian lady beetle Harmonia axyridis (Coleoptera: Coccinellidae) than ten other flower species (Adedipe & Park 2010). Visual factors such as color play a role in predator attraction, although in some species, visual responses tend to be nonspecific (Bernays & Chapman 2007).

In addition to visual cues, olfactory factors also play an important role. Flowering plants produce volatile compounds (VOCs) that act as chemical signals to attract natural enemies and shorten the time spent searching for food sources (Bramasta *et al*. 2023; Muhlison *et al*. 2025). In *Apolygus lucorum*, the combination of visual and olfactory cues enhances predator behavioral responses compared to single cues (Pan *et al*. 2015). Visual stimuli enhance olfactory responses to volatile compounds from host plants (Patt *et al*. 2011). Understanding the combination of visual and olfactory signals is crucial in determining *S. annulicornis* preferences for flowering plants.

In addition to attractiveness, the success of flowering plants in supporting predators also depends on the suitability of flower morphology to insect mouthparts (Parolin *et al*. 2012). Nectar accessibility is key to improving predator performance through additional energy intake (Winkler *et al*. 2009; Zhu P *et al*. 2014). Without appropriate access, visually and olfactorily attractive flowers will not provide functional benefits to predators (Wackers 2005; Van Rijn & Wackers 2016). Therefore, a comprehensive evaluation of flowering plant suitability should consider not only visual and olfactory preferences but also morphological compatibility between flowers and predator mouthparts. Accordingly, this study aims to (i) evaluate the visual and olfactory preferences of *S. annulicornis* toward selected flowering plant species, (ii) assess the morphological compatibility between the mouthparts of *S. annulicornis* and floral structures to support the conservation and biological control performance of *S. annulicornis* in agroecosystems.

2. Materials and Methods

2.1. *S. annulicornis* Rearing

Predator *S. annulicornis* was obtained from agricultural land and dry fields in Tamanarum Village, Patrang Subdistrict, Magetan Regency, East Java Province, Indonesia. The predatory insects obtained from the field were placed in rearing boxes, which would later be mated in a single box. These adult pairs were kept until they mated and produced eggs. The eggs produced were transferred to a different box to facilitate monitoring. The hatched instars will be placed in rearing boxes in groups according to the size of the rearing box, with a maximum of 20 individuals in a single rearing box measuring 17 × 17 × 8 cm (Truong *et al*. 2020).

2.2. Propagation and Maintenance of Insectary Flower Plants

The flowering plants used in the study met several criteria: they were commonly found across various ecosystems, had been documented in the literature, and their flowers had been shown to attract predatory insects. The eight flowering plant species used in this study were *Tagetes erecta*, *Arachis pintoi*, *Wedelia trilobata*, *Melampodium divaricatum*, *Lantana camara*, *Zinnia peruviana*, *Turnera subulata*, and *Turnera umlifolia*. Previous studies have demonstrated the potential of these species to attract natural enemies. For example, *Helianthus annuus* and *Tagetes erecta* have been reported to attract coccinellid predators (Adedipe & Park 2010), while *Lantana camara* and *Zinnia* spp. have been associated with increased abundance of predatory insects in agroecosystems (Chau *et al*. 2019). Other species, such as *Arachis pintoi* and *Wedelia trilobata*, have also been recognized for their role in supporting natural enemies (Jamian 2017; Hidayat *et al*. 2018). The flowering plant *T. subulata* has been reported to increase the survival and parasitization rate of egg parasitoid *Trichogramma* sp. (Usman *et al*. 2025). Flowering plants were obtained from seeds, which were then sown and cared for until they bloomed. Flowering plants were planted in polybags containing a growing medium composed of soil, compost, and rice husk in a 1:1:1 ratio. Plant maintenance was carried out through adequate watering and fertilization.

2.3. Visual Preference Test

This test was conducted using a cardboard box measuring 30 × 21 × 22 cm. This method draws on previous research, with adjustments to the types of insects and the availability of tools and materials (Adedipe & Park 2010). At the end of the cardboard box, an acrylic board was used, which would later be covered with yellow plastic film (RGB: 230, 217, 151), orange (RGB: 202, 160, 63), red (RGB: 191, 68, 74), blue (RGB: 76, 110, 151), green (RGB: 65, 119, 102), and white (RGB: 232, 221, 209). This method was adapted to the researcher's needs by adding partitions at each color boundary toward the back using 10 cm-long cardboard (Figure 1). This color preference test was repeated four times, with each repetition consisting of three males and three females to compare color preferences.

2.4. Olfactory Preference Test

The response of *S. annulicornis* to flowering plants was tested using an olfactometer following the method developed in previous studies (Camara Siqueira da Cunha *et al*. 2022; Usman *et al*. 2025). In this test, airflow was used as the olfactory stimulus in a Y-tube olfactometer. Each arm was connected to a container of the odor source in the form of a used gallon. Air was generated by an air pump (JEBO 9970 air pump) located at the end of the olfactometer tube. The odor source container was covered with a black lid to minimize color factors in the test. The airflow used in this study was four lpm (liters per minute) (Figure 2).

Olfactory preference tests were conducted without choice (non-choice) and with choice (choice) for each type of flowering plant. The non-choice test compared the use of olfactory cues from flowering plants with a control (clean air) to determine which flowering plants were attractants and repellents (Adedipe & Park 2010). The choice test involves comparing the use of one flowering plant with that of another to determine which is more attractive (Adedipe & Park 2010). In the non-choice test, four selected flowers were identified and then used in the choice test. Testing was conducted for 30 minutes per treatment, and insects were recorded as having made their choice upon crossing the finish line. If an insect had not made its choice within 30 minutes, it was not considered a respondent and had to be replaced with a new insect. Each treatment was repeated five times.

2.5. Measurements of *S. annulicornis* and Flower Morphology

The measurement method for *S. annulicornis* and the selected flower morphology was carried out with reference to previous research (Vattala *et al*. 2006). Based on the insect's size, measurements were taken using a digital caliper (Digital LCD Caliper, 0-150 mm range, ±0.01 mm accuracy). Measurements of predatory insects were taken after they were first killed to determine rostrum diameter and length. The depth of nectar availability was defined as the distance between the opening that could still be accessed by the rostrum of *S. annulicornis* (Van Rijn & Wackers 2016; Usman *et al*. 2025).

Figure 1. Visualization of the color preference test box of *S. annulicornis* for six test colors

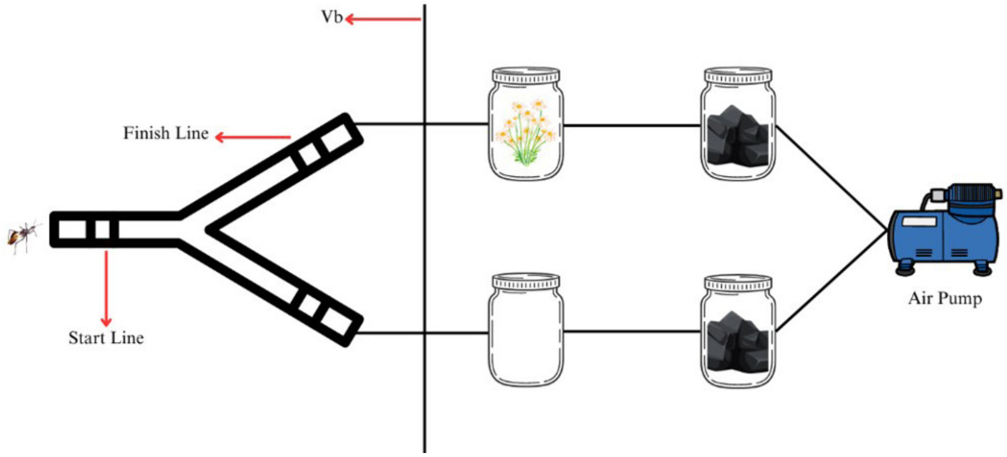

Figure 2. Visualization of the olfactometer device circuitry for testing the scent preferences of *S. annulicornis*. This testing circuitry begins with an air pump, activated charcoal, a scent source, and finally a Y-tube. Vb: black cover cloth; Finish line: determining line; and start line: line where the insect begins to be counted as walking

2.6. Data Analysis

Data from the visual test were analyzed using analysis of variance (ANOVA) in SPSS software. When the ANOVA results showed significant differences, Duncan's Multiple Range Test (DMRT) was conducted at the 5% significance level. Meanwhile, data from the olfactory test were analyzed using the chi-square (χ^2) test. This analysis was applied to both the non-choice and choice olfactory tests. All statistical analyses were performed in SPSS to obtain χ^2 values and their significance levels.

3. Results

3.1. Visual Preference *S. annulicornis*

Overall, white (2.00 ± 0.82^b) and yellow (1.75 ± 0.63^b) showed the highest individual preference, followed by blue, red, and green with lower preferences, while no individuals were observed to prefer orange (0.00 ± 0.00^a) (Figure 3). The presence of individuals across various colors indicates that the visual preferences of *S. annulicornis* are not restricted to a single color when selecting prey or habitat.

3.2. Olfactory Preference *S. annulicornis*

3.2.1. Non-Choice Test

T. erecta, *T. subulata*, *T. ulmifolia*, and *W. trilobata* have the highest visitation rates by *S. annulicornis* (82%, 80%, 73%, and 67%, respectively) with significant differences in preference ($p < 0.05$ and $p < 0.01$), indicating strong aromatic appeal. Conversely, *L. camara*, *Z. peruviana*, *M. divaricatum*, and *A. pintoi* showed low visitation rates (10%, 0%, 20%, and 30%) with significant differences ($p < 0.01$), indicating less-preferred olfactory cues (Figure 4).

T. erecta shows differences in preference based on sex, with males (100%) showing a significant difference ($p < 0.01$), while females (67%) show no significant difference ($p > 0.05$) (Table 1). These results indicate that host plant preferences are influenced by sex in accordance with biological needs such as food, oviposition, or mate searching. Females have a stronger preference for flowering plants because these plants provide additional food sources in the form of nectar, which supports their productivity.

3.2.2. Choice Test

T. subulata exhibited a significantly higher attraction to predators, as reflected by the greater proportion of visits (approximately 80%) compared to alternative odor sources (approximately 20%) (Figure 5), indicating the strong role of its olfactory cues. In contrast, *W. trilobata* had the fewest visits among plants, especially relative to *T. subulata* and *T. ulmifolia*, and showed results similar to *T. erecta* (73% vs. 27%, $p < 0.01$). *T. subulata* was the most attractive among the four tested plants, showing the highest selection percentage (100% in both males and females), and consistently outperformed the other tested flowers, with statistically significant differences indicated by the χ^2 test ($p = 0.002$) (Table 2). This is evidenced by *T. subulata* remaining superior to the three other test flowers. This result is further supported by the higher number of visits to *T. subulata* (26), compared

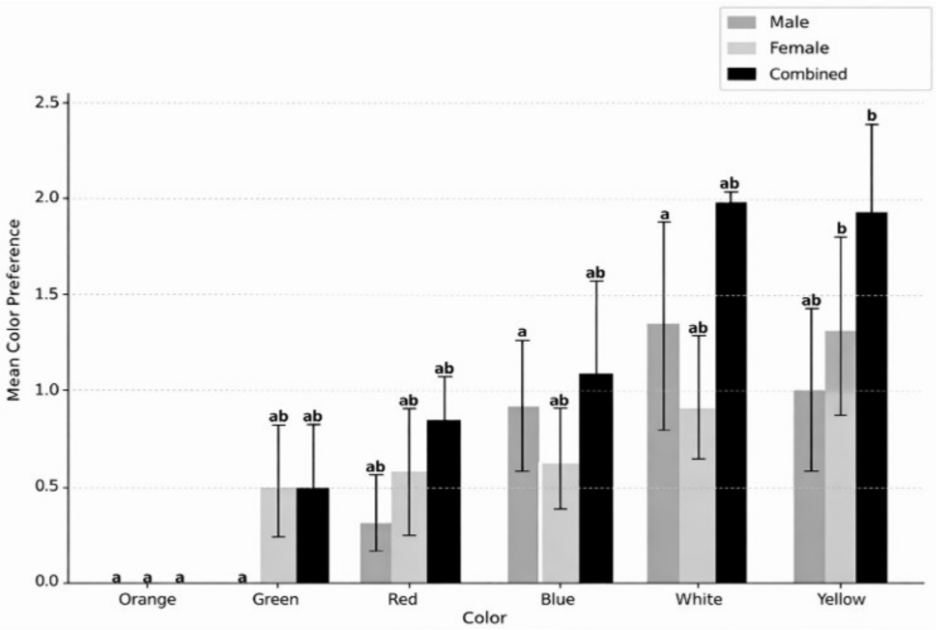

Figure 3. Average preference of *S. annulicornis* for various colors (orange, green, red, blue, white, and yellow). The notation on the bars indicates significant and insignificant differences in color treatment in each sex (male, female, and combined) (p<0.05)

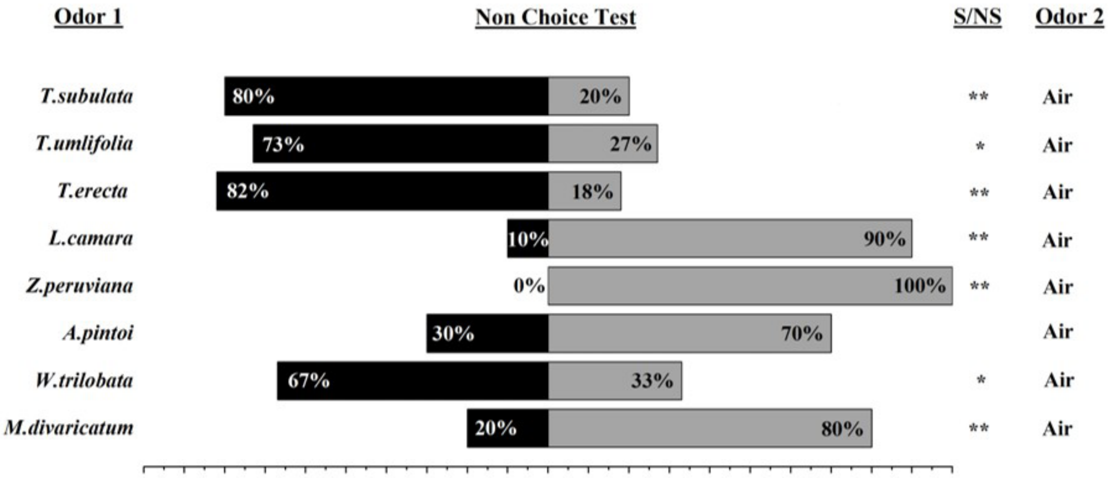

Figure 4. Total olfactory response of male and female *S. annulicornis* adults in the Non-Choice Test, measured based on the number of visits to each odor source. (** = p-value<0.01,* = p-value<0.05)

Table 1. Non-choice preferences of *S. annulicornis* for several flowering plant scents

Flowering plants	Male		Female	
	Flowering plants (%)	χ2 statistics (p-value)	Flowering Plant (%)	χ2 statistics (p-value)
T. subulata	80	0.058^{ns}	80	0.058^{ns}
T. umlifolia	80	0.058^{ns}	67	0.284^{ns}
T. erecta	100	0.002^{**}	67	0.284^{ns}
L. camara	20	0.058^{ns}	0	0.002^{**}
Z. peruviana	0	0.002^{**}	0	0.002^{**}
A. pintoi	20	0.058^{ns}	33	0.284^{ns}
W. trilobata	67	0.284^{ns}	67	0.284^{ns}
M. divaricatum	20	0.058^{ns}	0	0.002^{**}

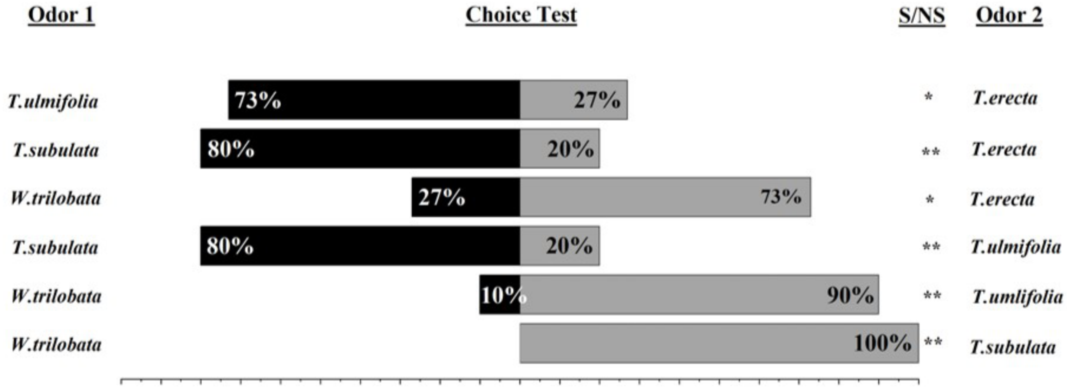

Figure 5. Overall olfactory response of male and female *S. annulicornis* adults in the Choice Test, measured based on the number of visits to each odor source. (**= p-value < 0.01; * = p-value < 0.05)

Table 2. Choice preferences of *S. annulicornis* for several scents of flowering plants

Treatment	Male			Female		
	Selected flowering plants	% Selected flowering plants	χ2 statistics (p-value)	Selected flowering plants	% Selected flowering plants	χ2 statistics (p-value)
T. umlifolia vs *T. erecta*	*T. umlifolia*	67	0.284^{ns}	*T. umlifolia*	80	0.058^{ns}
T. subulata vs *T. erecta*	*T. subulata*	80	0.058^{ns}	*T. subulata*	80	0.058^{ns}
W. trilobata vs *T. erecta*	*T. erecta*	67	0.284^{ns}	*T. erecta*	80	0.058^{ns}
T. subulata vs *T. umlifolia*	*T. subulata*	80	0.058^{ns}	*T. subulata*	80	0.058^{ns}
W. trilobata vs *T. umlifolia*	*T. umlifolia*	80	0.058^{ns}	*T. umlifolia*	100	0.002**
W. trilobata vs *T. subulata*	*T. subulata*	100	0.002**	*T. subulata*	100	0.002**

The table shows the results of testing the preferences of male and female insects for two types of flowering plants using the χ^2 (Chi-square) test. (** = p-value<0.01,* = p-value<0.05; ns: no significant difference)

to *T. umlifolia* (19), *T. erecta* (13), and *W. trilobata* (4) (Table 3).

3.3. Measurements of *S. annulicornis* and Flower Morphology

Measurements of *S. annulicornis* and flower morphology revealed that the rostrum dimensions of *S. annulicornis* were generally compatible with the floral structures of *T. subulata*, *T. ulmifolia*, and *W. trilobata* (Figure 6). This result indicates that the morphological compatibility between the predator and the floral structures may enhance feeding efficiency and support the ecological association between *S. annulicornis* and these flowering plants. In contrast, the floral structure of *T. erecta* appeared less compatible with the rostrum morphology of *S. annulicornis*. The average length of the rostrum (8.90 ± 0.10 mm) is significantly shorter than the average depth of the corolla (21.66 ± 0.35 mm) (Figure 6), suggesting a mismatch in structural dimensions that may limit effective nectar access. These observations indicate that morphological compatibility between predators' mouthparts and floral structures may influence nectar accessibility.

Table 3. Summary of the number of visits by *S. annulicornis* to selected flowering plants

Flowering plants	Number of visits
T. erecta	13
W. trilobata	4
T. subulata	26
T. umlifolia	19
Total	62

*The table shows the number of visits by *S. annulicornis* in all replicates and treatments. Calculations were based on combined data for males and females

4. Discussion

The present study demonstrates that *S. annulicornis* exhibits greater visitation and morphological compatibility with *T. subulata* than the other tested flowering species (Table 3, Figure 6), highlighting its potential as a suitable insectary plant for conservation biological control. The provision of flowering plants can enhance predator performance by supplying nectar as an additional food resource and shelter. Besides preying

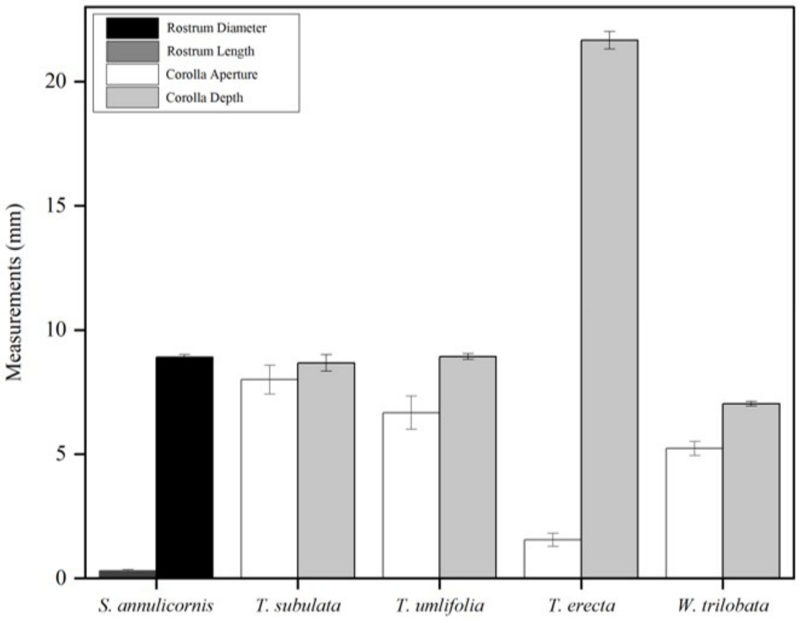

Figure 6. Morphometrics of *S. annulicornis* (rostrum diameter and rostrum length) and flowering plants (corolla aperture and corolla depth).

on insect pests, predators utilize nectar to increase their survival and activity (Portillo *et al*. 2012). Previous studies have shown that *Sesamum indicum* flowers can extend the lifespan of *Cyrtorhinus lividipennis* by two to three times (Zhu *et al*. 2014). Similarly, supporting the role of flowering plants in enhancing natural enemy performance, canola and niger flowers have been reported to increase populations of *Pristhecancus plagipennis* (Grundy & Maelzer 2003).

Multiple factors, including visual cues such as flower color, influence the presence of natural enemies on flowering plants (Schaller & Nentwig 2000), although color preferences vary among species (Bernays & Chapman 2007; Hung *et al*. 2025). In this study, *S. annulicornis* showed a tendency toward white and yellow flowers (Figure 3), although the differences were not statistically significant. Similar color preferences have been reported for other predators, including Harmonia axyridis and Hoverflies (Adedipe & Park 2010; De Luca & Vallejo-Marín 2013; Rodriguez-Saona *et al*. 2020), suggesting that bright colors function as long-range visual signals that enhance plant detectability within complex vegetation. Thus, visual cues may facilitate initial habitat location and resource orientation.

In addition to visual cues, floral scents or volatile organic compounds (VOCs) play an important role in attracting natural enemies (Schiestl 2010; Jamian *et al*. 2020). The composition of volatile compounds—particularly linalool, (E)-β-ocimene, and methyl salicylate (Fernandes *et al.* 2014), serves as a chemical attractant for natural enemies such as *Parapanteles hyposidrae* (Liu *et al*. 2024) and *Geocoris* spp. (He *et al*. 2019) and *Coccinella septempunctata* (Zhu, J. & Park 2005). These VOCs primarily function as short-range olfactory cues that guide predators toward nectar resources following visual detection. Consistent with this mechanism, *T. subulata* showed the highest level of attraction to *S. annulicornis*, followed by *T. ulmifolia*, *T. erecta*, and *W. trilobata* (Table 3). Compared to the other species, *T. subulata* produces a greater quantity and more complex types of VOCs, which are attractive to natural enemies (Hidayat *et al*. 2018; Jamian *et al*. 2020).

The synergy between visual and olfactory signals has been shown to increase the effectiveness of attractant plants (Pan *et al*. 2015; Gurr *et al*. 2017). Bright colors such as yellow and white help attract predators from a distance, while VOCs direct them toward the food source. However, accessibility to nectar remains crucial. *S. annulicornis*, with its piercing-sucking mouthpart type, requires a match between flower structure and rostrum length. Research findings indicate that *T. subulata*, *T. ulmifolia*, and *W. trilobata* exhibit morphological compatibility with *S. annulicornis*, whereas *T. erecta* does not (Figure 6). Previous research indicates that only flowers with a corolla depth <2 mm are effective in enhancing the survival of predatory and parasitoid insects (Vattala *et al*. 2006; Van Rijn & Wäckers 2016; Gontijo *et al*. 2013). The morphological compatibility of insects with flowers is crucial in determining nectar accessibility, which influences insect survival (Vattala *et al*. 2006; Usman *et al*. 2025).

The white color with a dark center in *T. subulata* supports the visual preferences of *S. annulicornis*, as Hansen *et al*. (2012) explain: visual patterns such as nectar guides increase foraging efficiency. In line with previous research, which showed that *T. subulata* is effective in attracting natural enemies in oil palm plantations, such as *Cosmolestes picticeps* and *Sycanus dichotomus*, compared to flowering plants like *Cassia cobanensis* and *Antigonon leptopus* (Jamian *et al*. 2017).

Volatile compounds and floral morphological compatibility likely enhance nectar accessibility for *S. annulicornis*, indicating that flowering plants serve not only as attractants but also as supplementary food sources that support predator performance. However, because this study was conducted under controlled conditions, the findings may not fully reflect the complexity of the field. Environmental variability and plant community interactions could influence predator responses, and further field validation is required.

Overall, *T. subulata*, followed by *T. ulmifolia* and *W. trilobata*, effectively attracted *S. annulicornis* and provided suitable nectar access. These results highlight the importance of considering floral attractiveness, nectar accessibility, and chemical traits when selecting plants for habitat management. The multifunctional role of *T. subulata* supports its potential use in flower strips or ecological engineering strategies to enhance biological control in agroecosystems.

In Conclusion, The results showed that the use of flowering plants *T. subulata*, followed by *T. ulmifolia* and *W. trilobata*, attracted *S. annulicornis*. Additionally, these plants are morphologically suitable for nectar access. Therefore, the role of flowering plants is not only as attractants but also as potential additional food sources accessible to *S. annulicornis*, enhancing its performance.

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