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Visual, olfactory, and nutritional stimuli of basil (*Ocimum basilicum* L.) in *Tetragonisca angustula* (Hymenoptera: Apidae)

Estímulos visuales, olfativos y nutricionales de la albahaca (*Ocimum basilicum* L.) en *Tetragonisca angustula* (Hymenoptera: Apidae

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ABSTRACT

Bees are essential pollinators that use visual and olfactory stimuli to locate flowers in search of nutritional resources. Among flowering plants, aromatics are recognized for their medicinal properties, but little is known about the mechanisms involved in their interaction with floral visitors. This work aimed to determine how visual and olfactory stimuli of the basil flowers participate in attracting *Tetragonisca angustula* Illiger (1806), and how microorganisms associated with these flowers are involved. For this, the attraction of bees was evaluated according to the type of flower and the resource offered (nectar, pollen, or nectar + microorganisms), and the bees' attraction to the plant's volatiles was analyzed, evaluating plants with and without flowers and the attraction to the isolated microorganisms in them. During the first phase (visual), *T. angustula* bees preferred white panicle-shaped flowers and the nectar resource. In the second phase (olfactory), they chose flowering plants over non-flowering ones. When plants were offered together with isolated microorganisms, bees preferred the smell of the microorganisms over the smell of the plants with flowers. The attraction of *T. angustula* to basil plants is influenced by the structure and color of the flowers, as well as by the volatiles, and especially by the presence of microorganisms in them, despite primarily seeking nectar as a resource during the visit.

Keywords: Epiphytic microorganisms; Insect behavior; Sensory cues; Semiochemicals; Stingless bees.

RESUMEN

Las abejas son importantes polinizadores que, mediante el uso de estímulos visuales y olfativos, localizan flores en busca de recursos nutricionales. Entre las plantas, las aromáticas se reconocen por sus propiedades medicinales, pero poco se conoce sobre los mecanismos que participan en la interacción con sus visitantes florales. Este trabajo tuvo como objetivo determinar cómo los estímulos visuales y olfativos de las flores de albahaca participan en la atracción de *Tetragonisca angustula* Illiger (1806) y cómo en esta interacción participan los microorganismos asociados a las flores. Para esto, se evaluó la atracción de las abejas de acuerdo con el tipo de flor y recurso ofrecido (néctar, polen o néctar + microorganismos) y se analizó la atracción de la abeja a volátiles de la planta, evaluando plantas con y sin flores, y la atracción hacia los microorganismos aislados en las mismas. Durante la primera fase (visual) las abejas de *T. angústula* prefirieron las flores blancas en forma de panícula y los recursos de néctar.; En la segunda fase (olfativa) eligieron plantas con flores sobre aquellas que no tienen. Cuando se ofrecieron las plantas junto con microorganismos aislados, las abejas prefirieron el olor de los microorganismos sobre el olor de las plantas con flores. La atracción de *T. angustula* a plantas de albahaca se ve influenciada por la estructura y color de las flores, así como por los volátiles emitidos y, especialmente, por la presencia de microorganismos en ellas, a pesar de que mayoritariamente buscan néctar como recurso durante la visita.

Palabras clave: Abejas sin aguijón; Comportamiento de los insectos; Microorganismos epífitos; Semioquímicos; Señales sensoriales.

INTRODUCTION

Bees are crucial in maintaining ecosystem functionality and stability (Ollerton, 2017). Through their interactions with plants, they not only secure essential food resources but also facilitate the pollination of a diverse array of flora, encompassing various shapes, colors, and sizes. This pollination process is crucial to produce many foods consumed by humans, underscoring the importance of conserving plant biodiversity (Klein *et al*. 2007).

Pollinating insects exhibit a range of social structures, from solitary lifestyles to complex communal systems (Michener, 2007). Among social bee species, *Apis mellifera* is the most recognized pollinator worldwide. Its prominence stems from its generalist foraging behavior, ease of management, and capacity for large-scale transportation, making it indispensable in commercial agriculture (Hung *et al*. 2018).

However, stingless bees, belonging to the tribe Meliponini, also play a vital role in pollination, especially in tropical regions. They are responsible for pollinating a substantial proportion of tropical plant species, both in native forests and agricultural settings (Slaa *et al*. 2006). Notably, *Tetragonisca angustula* (Illiger, 1806) is among the most utilized species in meliponiculture and serves as an effective pollinator for various plants, collecting essential nutrients such as proteins, minerals, fats, and oils through the gathering of water, nectar, and pollen (Ocaña-Cabrera *et al*. 2022).

Bees are highly attuned to visual and olfactory stimuli, which are essential for recognizing floral traits and navigating their environment (Giurfa, 2013). Visual cues, such as flower color and pattern, play a crucial role in long-distance attraction, enabling bees to effectively detect and discriminate between floral resources (Hempel de Ibarra *et al*. 2022). Among the reported stimuli for bees, those that generate the most significant attraction response at long distances are visual, such as the shape and color of flowers (Parra, 2001). Plant volatiles, which are compounds that give the olfactory profile of plants, play a significant role at short distances (Moré *et al.* 2010). In general, both visual and chemical (volatile) stimuli are signals of nutritional rewards, such as pollen or nectar, offered by plants to attract pollinators (Asociación española de Entomología, Jardín Botánico Atlántico y Centro Iberoamericano de la Biodiversidad, 2002).

In plants of the *Ocimum* genus, the presence of chemical compounds such as eugenol, linalool, trans-cinnamate methyl, geraniol, and thymol have been confirmed (Murillo Perea & Viña Patiño, 1999; Murillo *et al.* 2004; González-Zúñiga *et al.* 2011). Some of these compounds exhibit allelopathic effects, pesticide properties, or acaricide effects (Hüe *et al.* 2015; Silva Lima *et al.* 2018).

Pollinators, in the stage of resource recognition in plants, also interact with microorganisms present in the aerial part of the plants they visit, which can positively or negatively modify their behavior (Menzel, 2001). In plants, the habit of microorganisms also depends on the site they inhabit, as some are hosted in vascular tissues (endophytes) where they obtain enough resources to establish a mutualistic symbiosis and protect the plant (Germaine *et al.* 2004; Tsavkelova *et al.* 2007), while others remain in the aerial part or phyllosphere of plants (epiphytes) and are constantly exposed to abiotic factors and relationships with pathogens (Granados-Sánchez, 2003).

The mutualistic relationship between plants and pollinators is intricate due to plants' morphological and physiological variability, including flower parts such as sepals, petals, stamens, and carpels. These elements attract pollinators like bees, either through the resources they offer, such as nectar, pollen, oils, and resins, or through their colors, smell, shapes, and sizes (Aizen *et al.* 2002). In this interaction, the participation of microorganisms, as mentioned earlier, also plays a crucial role, resulting in either mutualistic or antagonistic relationships depending on the interacting agent (De Bruyner & Baker, 2008).

Therefore, the aim of this study was to elucidate the factors involved in the bee-plant interaction, evaluating visual, olfactory, and nutritional stimuli, as well as possible associated microorganisms that participate in the selection of plants by *Tetragonisca angustula*.

MATERIALS AND METHODS

To evaluate the behavioral responses of *Tetragonisca angustula* to stimuli associated with white basil plants, fifty cuttings of the lemon variety (*Ocimum basilicum* L. 1753) were planted in rooting trays. These plants were kept in trays with peat as a substrate for 15 days. After this period, the cuttings were transplanted into polyethylene bags. A mixture of river sand, rice husk, and worm humus in a 1:1:1 ratio was used as substrate for transplantation. The polyethylene bags were placed in a greenhouse (3.96 m long, 3.83 m wide, 2.93 m high) in the experimental farm of the Universidad del Magdalena in Santa Marta (N 11°13' 24", W 74°11' 2.3").

Plants were enclosed in white mesh boxes to prevent insect damage that could alter metabolites emitted by the selected plants for olfactory stimulus analysis. Additionally, a single fertilization with triple 15 (NUTRIMON) (dose: 5 grams per plant) was performed 10 days after transplantation. Finally, the plants were ready for the bioassays. According to the Köppen-Geiger classification (1961), the area where this study was conducted corresponds to BSh local steppe climate.

Recognition and extraction of microorganisms associated with basil plants. To identify epiphytic and/or endophytic microorganisms associated with basil flowers (*Ocimum basilicum),* isolations were carried out in the plant pathology laboratory of the Universidad del Magdalena. In this study, 40 complete basil flowers were selected. Four isolation treatments were established using different floral structures to evaluate the presence of microorganisms: complete flowers with superficial washing with sterile water (Treatment 1), macerated washed complete flowers (Treatment 2), unwashed petals (Treatment 3), and unwashed stamens (Treatment 4). Two replicates of each treatment were performed using five flowers. All plant material was previously washed with distilled water. The procedure for Treatment 2 was based on the methodology of Stone *et al.* (2004) and Photita *et al.* (2004).

After planting each structure on nutrient agar, two purifications were carried out for each treatment using four types of agars: nutrient agar (Purification 1), potato nutrient agar (Purification 2 and 3), and soy nutrient agar (Purification 4). Purifications were carried out using the streak plate method for bacteria and direct seeding for fungi (Clarke *et al*. 2018).

Microbiological sample analysis. An initial seeding was carried out, and from there, purifications were performed until obtaining the pure strain in each Petri dish. The samples were identified through microscope mounts, using Gram staining for bacteria and lactophenol blue staining for fungi. The physical data of each sample, such as color, shape, elevation, and margin, were recorded. This was done to identify the present fruiting bodies, following an illustrated guide to fungi and plant pathology (Koneman & Dowell, 1989; Cañedo & Ames, 2004; Agrios, 2005).

Behavioral Bioassays. Stage 1. Evaluation of learning. The bees used to evaluate their behavioral response to each stimulus were taken from the meliponary of the experimental farm of the Universidad del Magdalena. They were kept isolated in their hive within a greenhouse (3.96 m long, 3.83 m wide, 2.93 m high), mounted on a wooden structure (80 cm wide, 160 cm long), preventing them from having previous olfactory and/or visual experiences associated with basil plants that were being cultivated nearby for this specific experiment. Before evaluating the bees' attraction to basil plant stimuli, they underwent training in an observation arena to establish the foraging area, where the resources to be evaluated would be offered. For this stage, the hive was placed at one right end corner of the wooden structure inside the greenhouse. Two Petri dishes with a 1:1 water solution with sugar (changed daily) were placed in the other right corner and in one left corner. Observations were made, measuring the number of visits for four hours (9:00 am -11:00 am and 3:00 pm -5:00 pm) for five days to assess the bees' learning level towards that stimulus and understand their response to the food source. After the learning phase, activities were carried out to evaluate stimuli provided by the basil plant.

Stage 2. Evaluation of visual stimuli. Nine treatments were evaluated in the observation arena, using three flower shapes (emulating form to panicle, solitary flower, simple leaf) and three colors (white, green, and purple), made with Eppendorf tubes filled with gauze with a sugar-water solution (1:1) and surrounded by foam to emulate the basil flower, with a size of 4 cm in height, 7 cm in diameter. Treatments to determine visual stimuli (color and shape) were distributed as follows; for treatments 1, 2, 3 (control), purple flowers were used in the three previously described forms, for treatments 4, 5, 6, green flowers were used, and for treatments 7, 8, 9, white flowers were used. Each treatment had ten repetitions in the observation arena, and five experiment replicates (each replicate representing one day of setup). In each replicate, the treatments were position changed to eliminate the possible influence of abiotic factors and the learning factor associated with the location on the insect's behavioral response. Observations were made during three periods of the day (7:00 am – 9:00 am, 11 am - 1:00 pm, 3:00 pm – 5:00 pm). The bee's arrival at the flower, followed by the extension of the glossa for feeding, was considered a positive response to the stimulus.

Stage 3. Association of nutritional rewards with visual stimuli of basil plants. Starting from the visual stimulus with the highest number of visits obtained in stage 2 and following the methodology from the previous stage, nine treatments with ten repetitions in the observation arena and five replicates (days of assembly) were established. The aim was to observe the relationship between the bees' visit and the nutritional reward offered by the plant (pollen or essential oil), including two microorganisms frequently isolated from the floral structures of basil in the treatments. This aimed to evaluate their effect on attraction to the plant. Nine treatments were designed with three attractant bases: (1) T1-T3 used water + sugar (1:1), with T1 as control, T2 adding *Aspergillus* sp.1, and T3 adding *Aspergillus* sp.2; (2) T4-T6 replaced the base with basil essential oil extracted from basil leaves (T4:control, T5: +sp. 1, T6: +sp.2); (3) used pollen solution (T7:control, T8: +sp. 1, T9: +sp.2) maintaining the same microorganism sequence across group.

The extraction and preparation of the solutions of the two microorganisms involved taking two strains, each with a different morphology but belonging to the same genus (*Aspergillus* sp.). To each strain, 10 ml of sterile water was added to remove all the fruiting bodies to the culture medium using a microbiological loop. The material was gently scraped, deposited in two 80 ml beakers, covered with aluminum foil, and then the conidia count was performed using the hemocytometer test with the Neubauer chamber (Vélez *et al.*1997). This determined the concentration of conidia in a suspension, obtaining the same quantity of conidia/ml for each microorganism. The treatments with microorganisms were standardized based on a concentration of 1,000,000 conidia/ml, achieved by adding 0.06 ml and 0.08 ml of the mother solution of microorganisms 1 and 2, respectively.

For the treatment with pollen-supplemented flowers, daily collections of 120 inflorescences provided pollen for treatments T7-T9. After anther excision: T7 received 1 mL sterile water (control), T8 was supplemented with 1 mL *Aspergillus* sp.1 suspension (1×10^6 conidia/mL), and T9 with 1 mL *Aspergillus* sp.2 suspension (equal concentration), all deposited in individual Eppendorf tubes with standardized pollen quantities. Finally, for treatments involving the incorporation of essential oils, 1 ml of ml extract was obtained from basil cuttings using 96% ethanol, excluding the floral area. It is important to note that this extract may not consist solely of essential oils, but rather a mixture of compounds, including both volatile and non-volatile substances extracted by ethanol.

Evaluation of olfactory stimuli. To assess the responses of the angelita bee (*T. angustula*) to olfactory stimuli from basil plants (*Ocimum basilicum*), an air flow olfactometer was designed following the methodology described by López-Ávila & Rincón (2006). The system circulated air provided by a compressor, which passed through an activated charcoal filter to ensure the cleanliness of the air entering the system. After the adaptation and establishment of the olfactometer located in a greenhouse, the behavior of the bee was evaluated concerning different olfactory stimuli from basil. The Bees used in the experiments were taken from a hive belonging to the Meliponario of the Universidad del Magdalena. These bees had no prior training and were taken directly from the hive entrance.

The olfactometer was adapted for choice experiments to use only two chambers (Figure 1), each containing different odor sources according to each treatment. The air flow laminar velocity in each arm was calibrated using anemometers (AMPROBE TMA-20HW). The arms were covered with black fabric to prevent bees from having an additional stimulus other than olfactory.

Figure 1. Olfactometer adapted with two chambers to study the interaction between *Tetragonisca angustula, Ocimum basilicum*, and the microorganisms associated with *O. basilicum*.

Initially, the attractiveness of basil plants in vegetative development or flowering phase was analyzed using the following treatments (phase 1): T0 = Plant without flowers (Arm 1) vs. pure air (Arm 2), T1 = Basil plant with flowers (Arm 1) vs. pure air (Arm 2), and T2 = Basil plant with flowers (Arm 1) vs. basil plant without flowers (Arm 2).

Considering the results of the previous stage, we proceeded to analyze whether the bee's attraction to the plant is generated by the mixture of volatiles emitted by the plant and its associated microorganisms, or only by the volatiles emitted by one of the microorganisms found in greater proportion in the floral structures of the plant (phase 2). The treatments always contrasted the attraction of the selected plant vs. the microorganisms as follows: T0: Culture medium with microorganism 1, T1: Culture medium with microorganism 2, and T2: Culture medium with microorganisms 1 and 2.

For each treatment, 20 bees were used as replicates, evaluated between 9:00 am and 12:00 pm, considering this as the time with the highest activity of *T. angustula* according to the learning evaluation results (stage 1). Bees were introduced one at a time, disconnecting the pump and placing the individual in the center of the olfactometer. After reconnecting the pump, the female received the stimulus, and it was observed until it chose one of the two arms or for a maximum of five minutes, after which it was removed. After removing each bee, the tube was cleaned with 70% alcohol to eliminate any possible traces left by the previous individual. Subsequently, it was allowed to dry before testing the next bee. The odor sources (treatments) were changed to the opposite arm after testing five individuals to correct any unforeseen asymmetry in the experimental setup. At the end of each treatment, the system was cleaned with hexane, allowing it to pass through the cylinders and arms for 20 minutes, avoiding any interference from residual odors from previous treatments.

Statistical analysis. To analyze bees' behavioral response to visual and nutritional stimuli and evaluate their learning response, a generalized linear model (GLM) with a Poisson distribution (Link = log) was used, adjusting the error distribution with quasi-Poisson distribution when necessary. The explanatory variables were the time of day and nutritional treatments. A contrast analysis within a GLM was conducted for each level of these variables to assess differences between the means of the explanatory variables.

A generalized linear model (GLM) with a binomial distribution (link = logit) was employed for the attraction response of bees to volatiles from both plants and microorganisms. The explanatory variables were the phenology plant stages and the microorganisms from the isolation phase, and the evaluated variable was the attraction response of *T. angustula*. The significance of the variables in all analyses was obtained as follows: first, we compared complex models with simpler ones obtained by combining levels of the variables; if simplification did not result in significant changes, the simpler model was accepted, maintaining the principle of parsimony (Crawley, 2012). After evaluating all models, they underwent a residual analysis to verify that the model was appropriate and that the error distribution was normal. For all analyses, an $\alpha = 0.05$ was considered to assess the statistical significance of variables. All analyses were conducted using R software (R Development Core Team, 2020).

RESULTS AND DISCUSSION

Recognition and extraction of microorganisms associated with basil plants. After seeding and purifying floral structures in culture media, fungi were the predominant microorganisms, and some bacteria were also found. The fungi isolated belonged to the *Aspergillus* genus. Therefore, only two morphotypes of the *Aspergillus* genus were purified, occupying up to 90% of the Petri dishes in the microbiological cultures used. During isolation, only the presence of epiphytic microorganisms in basil flowers was identified. However, the presence of endophytic microorganisms is not ruled out, given the findings of treatments derived from macerated flowers.

The microorganisms in ecosystems establish various relationships, ranging from synergies to antagonisms, as well as physical and biochemical competitions. These interactions are regulated by a series of factors, both biotic and abiotic (Cano, 2011). Microorganisms have a wide range of hosts and are isolated from seeds, flowers, leaves, and stems. Among the most common are endophytes (such as *Pseudomonas* and *Bacillus*), categorized as activators of defense mechanisms in the plant, as well as epiphytes (such as *Alternaria* and *Aspergillus*), which can be pathogenic (Hallmann *et al.* 1997; Rosenblueth & Martínez-Romero, 2006; Acosta *et al.* 2005). This diversity of microorganisms supports the finding of the fungus *Aspergillus* sp.1 and *A*. sp. 2, epiphytic microorganisms obtained from basil flowers. Additionally, the presence of endophytic microorganisms is evident in other aromatic plants; for instance, *Bacillus subtilis* has been isolated from *Mentha spicata* tissues, demonstrating its role in promoting plant growth and enhancing resistance to pathogens (Castro-Restrepo *et al*. 2022).

The plant material used is related to previous stages of observation and recognition of the structures that *T. angustula* visited most frequently. Bee training showed that the ability to recognize a food source is positive and significant based on the number of visits they made. It is worth noting that foraging times for obtaining rewards vary depending on the resource offered, with the peak search for pollen, oils, and resins occurring in mid-morning (8:00 am – 10:45 am), while at the beginning and end of the afternoon (11:00 am – 5:00 pm), bees prefer nectar, as suggested by Álvarez *et al.* (2014) with *Bombus* sp. and *Thygater analis*, and Torretta *et al.* (2010) with *Melissoptila* sp., *Melissodes* sp., and native bees. Their period of greatest activity is from 8:30 am to 11:45 am (Torretta *et al.* 2010; Carmona-Diaz *et al.* 2017).

Evaluation of Learning. The significant increase in feeder visits across training days (stage 0) (F (1,8) = 120.91, P = 1.14×10^{-5}) demonstrated bees' capacity for associative learning, as bees progressively improved at recognizing the food stimulus location. The absence of time-of-day effects (F(1,7) = 3.52, P = 0.10) further confirmed that this learned response was specifically tied to spatial cues rather than temporal patterns. Together, these results (Figure 2) establish that bees successfully formed and retained a location-based association with the food reward through repeated exposure.

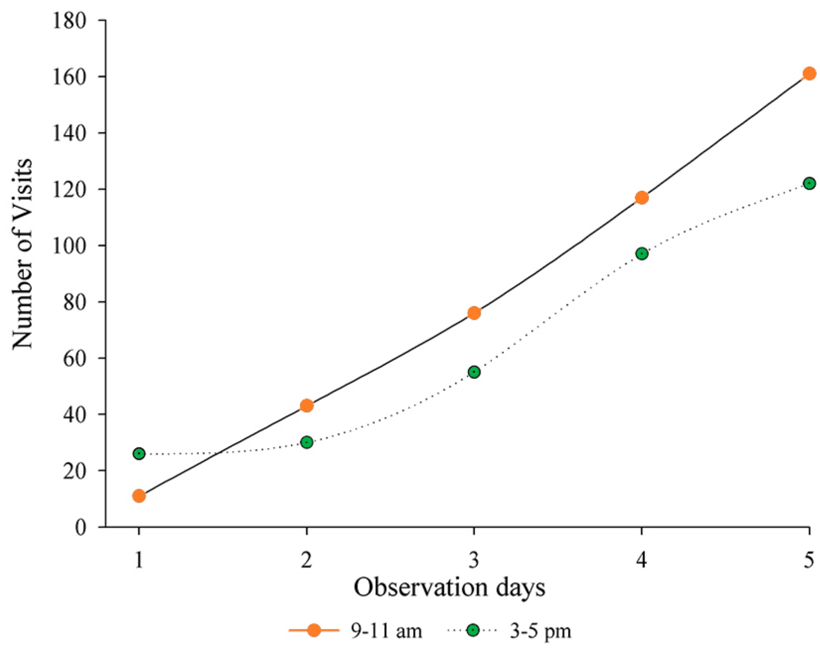

Figure 2. Number of visits of *Tetragonisca angustula* to the training feeders during the five days of observation according to the time of day (F (1,8) = 120.91, P= 1.144e-05).

The preference of bees for a resource and the frequency of their visits depend on the nutritional requirements of these insects, as well as the availability, quality, and quantity of resources that a plant offers them (García-García *et al.* 2001, Woodcock *et al.* 2014). *T. angustula* is a generalist bee that visits numerous plant species in search of resources, including botanical families such as Euphorbiaceae, Anacardiaceae, and Rutaceae (Carvalho & Bego, 1995). This bee shows a preference for flowers that offer a greater quantity of the resource it needs. For example, it has an affinity for basil, selecting flowers in a panicle arrangement, which aligns with the findings of Lopes de Carvalho *et al.* (1999). In addition to basil, other frequent sources of pollen and nectar for *T. angustula* can be *Bulbine frutescens*, *Eucalyptus* spp., and *Leucaena leucocephala*, which share structural and resource similarities with basil (Lopes de Carvalho *et al.* 1999).

Association of nutritional rewards with visual stimuli from basil plants. Throughout the evaluation process, a total of 6604 visits were recorded, with the flower shape influencing bee visits ($F_{(8,126)} = 49.73$; $P<2.2e^{-16}$) (Figure 3). In this case, the number of visits did not vary by the time of day either ($F_{(2,124)} = 0.55$; $P=0.57$). Bee responses were influenced by the color of the flower ($F_{(8,126)} = 49.66$; $P<0.0001$); thus, treatment 7, with a feeder resembling a panicle and white in color, showed the highest number of visits, followed by treatments 1 and 4, both also resembling a panicle and being purple and green, respectively. These two treatments had the same attraction effect ($F_{(6,124)} = 0.18$, $P= 0.67$). Treatments T2, T6, T8, T9 exhibited the same attraction response ($F_{(3,130)} =0.96$, $P= 0.33$), and treatments T3 and T5 showed the same effect ($F_{(6,127)} =0.102$, $P=0.75$) and the lowest number of visits (Figure 4).

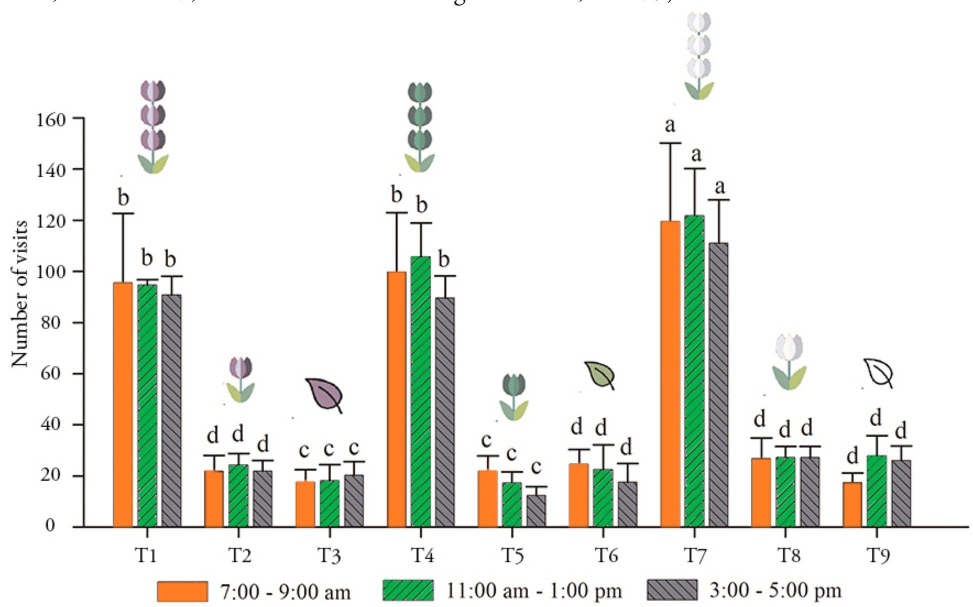

Figure 3. The average number of *Tetragonisca angustula* visiting feeders according to their shape and color.
T1: purple panicle, T2: purple solitary flower, T3: purple single leaf, T4: green panicle, T5: green solitary flower, T6: green single leaf, T7: white panicle, T8: white solitary flower, T9: white single leaf.
Bars with different lowercase letters have significant differences according to the contrast analysis of means within the Generalized Linear Models (GLM) with α <0.05.

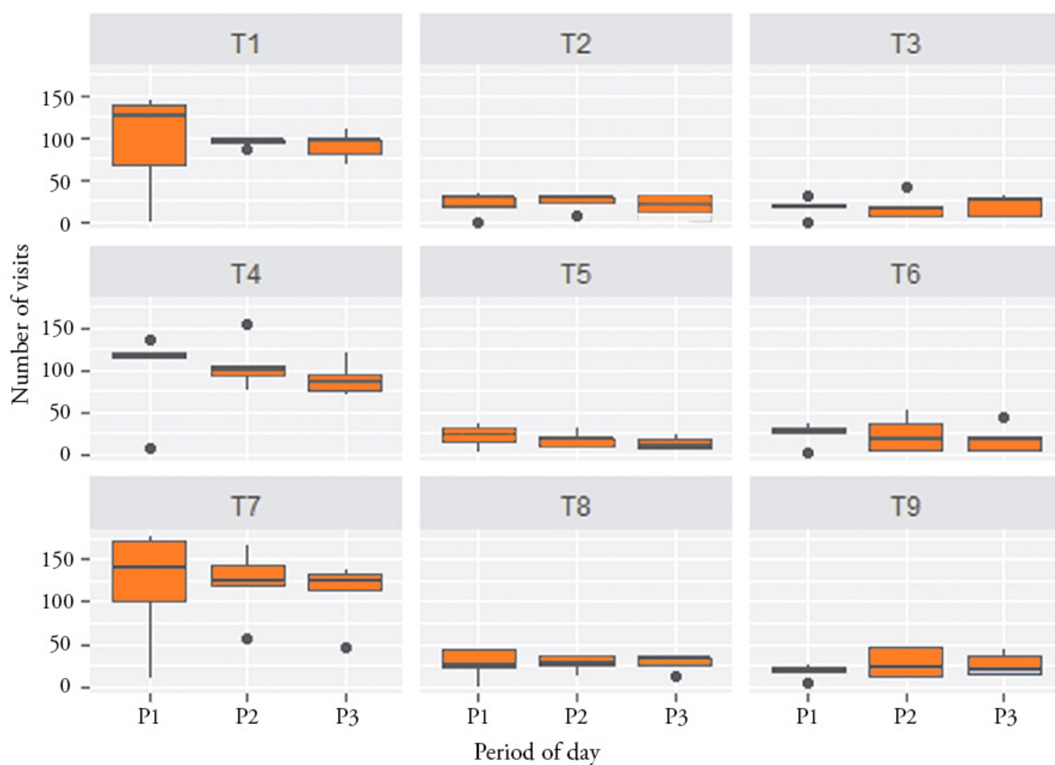

Figure 4. Number of *Tetragonisca angustula* visiting feeders according to treatment and time of day.
T1: Purple panicle, T2: Purple solitary flower, T3: Purple single leaf, T4: Green panicle, T5: Green solitary flower, T6: Green single leaf, T7: White panicle, T8: White solitary flower, T9: White single leaf.
Period of day: P1= 7:00-9:00 am, P2= 11:00-1:00 pm, P3= 3:00-5:00 pm.

According to these results, artificial flowers preferred by bees were multiplied, corresponding to the white color with a panicle shape. In this phase, a total of 3106 visits were recorded, showing a significant preference for the control treatment (supplemented with water + sugar) with a higher number of visits during the periods of 11:00 am – 1:00 pm (P2; figure 5b) and 3:00 – 5:00 pm (P3; figure 5c). In the first period of the day (P1: 7:00 - 9:00 am; figure 5a), more visits were observed in flowers with essential oil and with sugar solution and microorganism 1 (T3 + T4). According to the observations of the visits, there are significant differences in the early morning hours (P1) and the periods at the beginning and end of the afternoon (P2 and P3) ($F_{(3,131)} = 73.36$, $P=2.80e^{-16}$), but there is no difference in the number of visits between the latter ($F_{(3,126)} = 1.91$, $P=0.13$). The response of the insects varies according to the offered food resource ($F_{(8,126)} = 18.49$, $P < 2.2e^{-16}$).

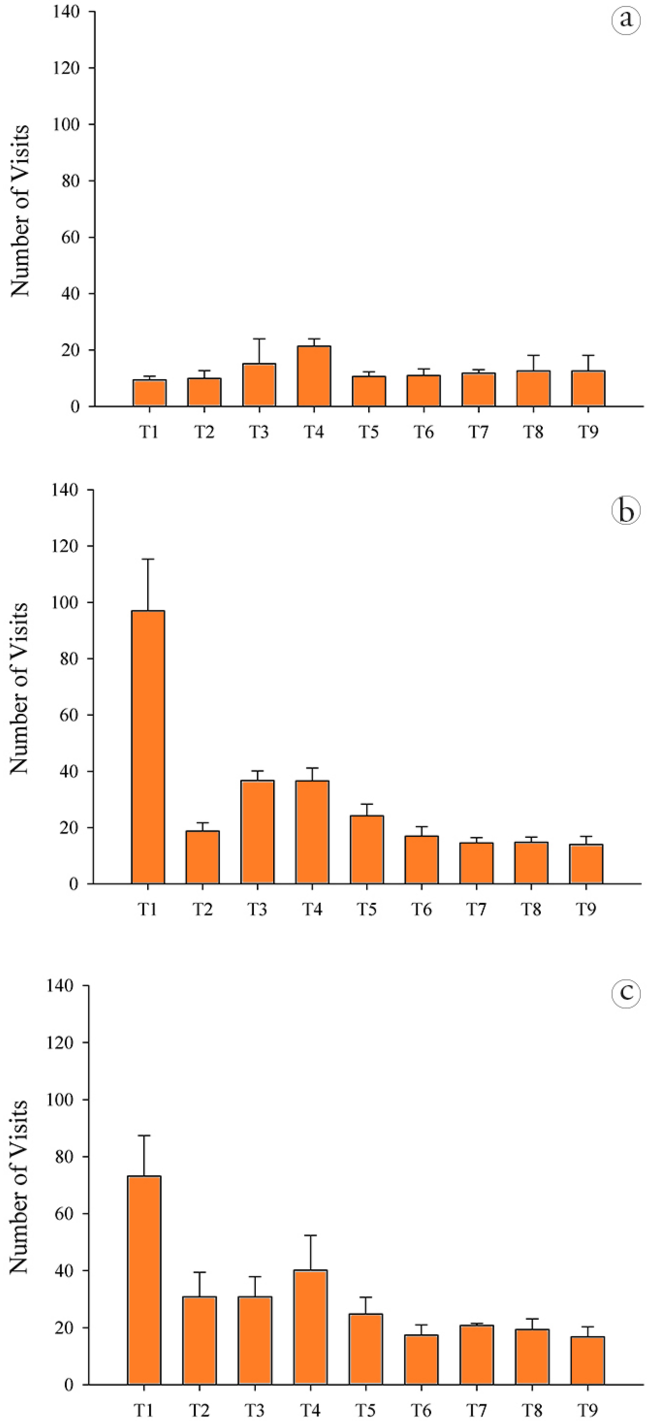

Figure 5. The average number of *Tetragonisca angustula* visiting feeders according to the food resource offered and time of day; a) P1= 7:00-9:00 am; b) P2= 11:00-1:00 pm; c) P3= 3:00-5:00 pm.

T1: flowers with water + sugar solution (1:1), T2: flowers with water + sugar + Microorganism 1 (Aspergillus sp.1), T3: flowers with water + sugar (1:1) + Microorganism 2 (Aspergillus sp.2), T4: flowers with essential oil, T5: flowers with essential oil + Microorganism 1 (Aspergillus sp.1), T6: flowers with essential oil + Microorganism 2 (Aspergillus sp.2), T7: flowers with pollen solution, T8: flowers with pollen solution + Microorganism 1 (Aspergillus sp.1), T9: flowers with pollen solution + Microorganism 2 (Aspergillus sp.2). A significant preference (p<0.05) was observed for times B and C of visits to the supplemented treatment of sugar water, followed by T3, T4 treatments in the three times evaluated.

Bees, in their search for rewards offered by plants, acquire information through their stimuli based on experience related to them, where their visual learning ability represents what is known as adaptive behavioral response (Avarguès-Weber *et al.* 2020). Srinivasan *et al.* (1994) demonstrated that previous visual experience can speed up image processing, allowing bees to categorize visual patterns and distinguish shapes and colors. In addition, they can recognize different sensory stimuli simultaneously with the visual ones. These findings support the positive learning response observed in bees in this study, as well as, their ability to recognize the food source (Srinivasan *et al.*1998; Giurfa *et al.* 1996).

This genus of fungi plays an essential role in biotechnology and decomposition of organic matter; furthermore, it is recognized as one of the most abundant in nature that can be found in various environments (García-Conde *et al.* 2024). It is also recognized by including important species that maintain beneficial and pathogenic relationships (Dagenais & Keller, 2009; Foley *et al.* 2014). Foley *et al.* (2014) examined beehives to determine the pathogenicity and virulence of common isolates of *Aspergillus*; the results showed that *A. flavus*, *A. nonius*, and *A. phoenicis* were pathogenic to larvae of *Apis mellifera carnica* (Pollman), and adults showed a high susceptibility to infection.

Taking this into account, the attraction of *T. angustula* was evaluated through tests with flowering plants and artificial flowers, along with isolated microorganisms. Diluted solutions of these microorganisms were also tested with other resources (water + sugar, essential oil, pollen, microorganisms alone). The results revealed that during training with artificial flowers (considering both visual stimuli and rewards), bees preferred solutions of sugar water (nectar) + *Aspergillus* 1 and essential oil + *Aspergillus* 2. However, when evaluating only the olfactory response, bees showed no preference for compounds emitted by *Aspergillus* sp. 1. A non-significant response was also observed when the smell of *Aspergillus* sp. 2 was offered as an alternative. However, one arm containing combined volatiles of both microorganisms (*Aspergillus* sp.1 and sp.2) against the opposite arm with flowering basil volatiles, *T. angustula* displayed significantly higher attraction to the microbial volatile mixture than to floral volatiles alone.

Olfactory stimuli evaluation. When evaluating the olfactory response of bees in relation to the plant's phenology, it was evident that: when individuals were given the choice between stimuli from plants without flowers and pure air, 30% of the evaluated individuals responded to the stimulus from plants in the vegetative state (without flowers). When individuals were given the choice between stimuli from flowering plants and pure air, 45% of the evaluated individuals responded to the stimulus from plants in the flowering state. When individuals were given the choice between stimuli from plants in vegetative state and plants with flowers, it was observed that 70% of the evaluated bees preferred the olfactometer arm containing the flowering basil plant (Figure 6a).

Upon evaluating the preference response of *T. angustula* towards basil plants with flowers (plants with higher attraction in phase 1) and the isolated microorganisms, it was observed that when individuals were exposed to the stimulus from *Aspergillus* sp. 1 and the stimulus from basil plants with flowers, 90% of the evaluated individuals preferred the arm containing the flowering plant. When the stimulus came from flowering plants and *Aspergillus* sp. 2, 65% of the evaluated individuals responded to the stimulus from flowering plants. However, when the stimuli came from flowering plants and the two microorganisms (*Aspergillus* sp. 1; *Aspergillus* sp. 2), 75% of the individuals preferred the stimulus from the two microorganisms (Figure 6b).

Bee visits to a plant are influenced by various stimuli, including olfactory cues. It is known that smells emitted by plants are determined by the production of volatile compounds (Calín-Sánchez *et al.* 2012), which vary according to the plant's structure (stems, leaves, or flowers) (Chalchat & Özcan, 2008; Jiang *et al.* 2016). In the case of basil, it produces essential oils whose chemical composition varies depending on the source structure. Jiang *et al.* (2016) found quantitative and qualitative differences in the compounds emitted by the leaves (β-pinene, Limonene, and Cedrene) and the flowers (β-pinene, α-bergamotene). Additionally, they determined that *O. basilicum* flowers emit compounds at a higher rate than the leaves, including monoterpenoids such as β-pinene and sesquiterpenoids such as α-bergamotene.

The results show that the flowering phase of *O. basilicum* is the most attractive stage to *T. angustula*, considering the choice of plants with flowers over plants without flowers. This suggests that the volatiles emitted by basil plants could help direct *T. angustula* in its preference for reward sources available in different phenological stages. The choice of these bees towards flowering plants is determined by the diversity of semiochemical substances emitted in the floral phase and their concentrations (Matu *et al.* 2021). However, when presented with other olfactory sources, such as pure air, *T. angustula* did not prefer basil plants, as observed in the olfactometer control treatments.

Despite field observations indicating that *T. angustula* visits *O. basilicum* plants, a clear preference for this plant is not evident, as reflected in the results; the effective visits of these bees to basil seem to be conditioned by the interaction of visual and olfactory factors. The latter could be influenced by the presence of other microorganisms in the plant (Vannette, 2020). This is supported by the findings of microbial isolation, revealing the predominant presence of two epiphytic morphotypes of the genus *Aspergillus*, found in both petals and complete flowers of *O. basilicum*.

It's worth mentioning that microorganisms emit various volatile substances (Steiner *et al.* 2007; Davis *et al.* 2012; Dickschat *et al.* 2018). For example, *Aspergillus versicolor* Vuillemin emits sesquiterpenes (Sunesson *et al.* 1995; Steiner *et al.* 2007; Dickschat *et al.* 2018). These emissions play a crucial role in the behavior of some insects. Davis *et al.* (2012) studied the volatile emissions of epiphytic fungi on apples as semiochemical attractants for eusocial wasps, demonstrating that volatiles emitted by the epiphytic fungus *Aureobasidium pullulans* can influence the attraction of these wasps, specifically *Vespula pensylvanica* and *V. germanica*, which are oriented towards these crops.

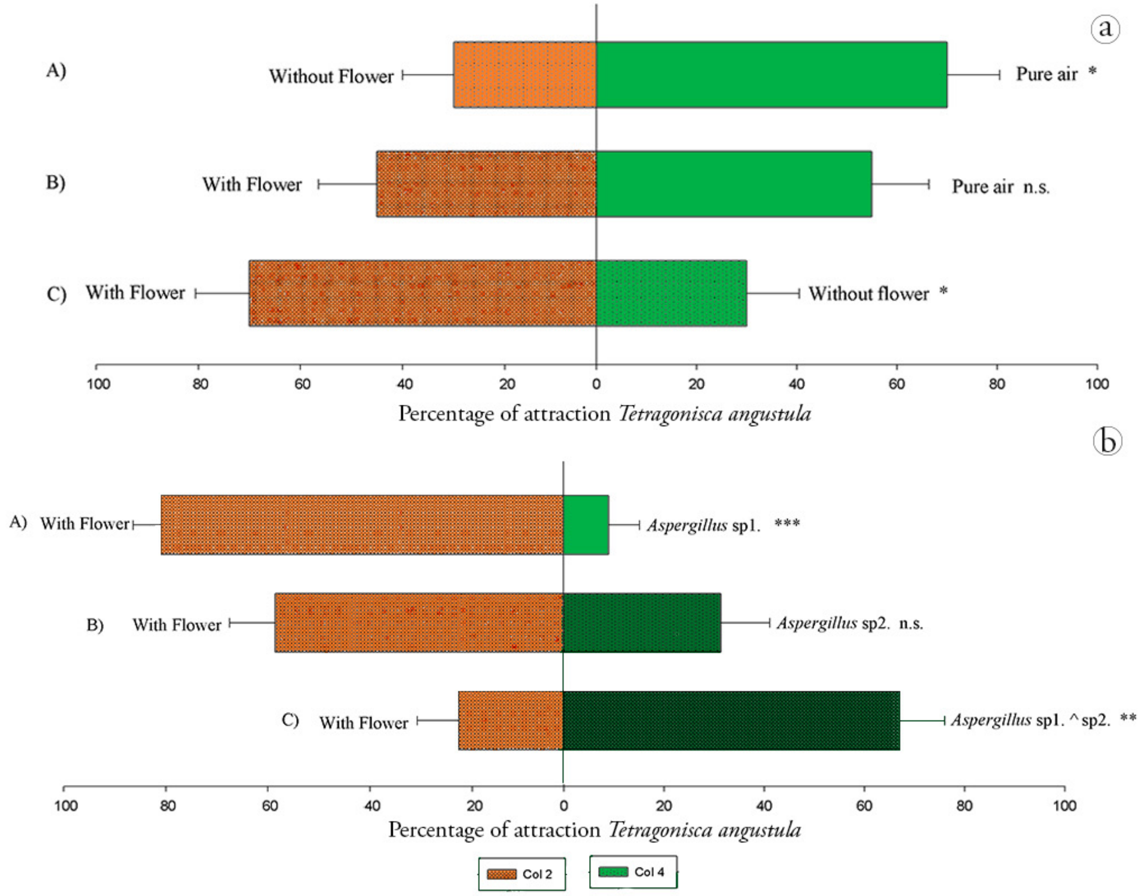

Figure 6. a) Percentages of net attraction (± SE) of *T. angustula* to basil plants. A) plants without flowers/pure air $\chi 2(1,38) =48.869$; $P=0.0103$; B) flowering plants/pure air $\chi 2(1,38) =55.051$; $P=0.5267$; C) flowering plants/non-flowering plants $\chi 2(1,38) =48.869$; $P=0.0103$. b) Percentages of net attraction (± SE) of *T. angustula* to volatiles emitted by isolated microorganisms. A) flowering plants/*Aspergillus* sp.1, $\chi 2(1,38) =26.007$; P= 5.752e-08; B) flowering plants/*Aspergillus* sp.2, $\chi 2(1,38)$ =51,796; $P=0.05587$; C) flowering plants/ *Aspergillus* (sp.1 and sp.2) $\chi 2(1,38) =44.987$; $P=0.001217$.
*Indicates significant differences ($P<0.05$) in comparisons.

Therefore, it is suggested that *T. angustula* bees may be guided by obtaining a resource along with volatiles emitted by these *Aspergillus* microorganisms, and that the variation in the volatile profile of each morphotype may be crucial for responses, whether attraction or non-preference. This considers that microorganisms produce multiple volatile organic compounds, and insects are oriented towards a mixture of compounds representative of fungal volatiles, which vary in their chemical composition, with some being more attractive than others (Steiner *et al.* 2007; Davis *et al.* 2012).

The results demonstrate that the smell of flowers and microorganisms associated with *O. basilicum* is essential for the interaction with *T. angustula*. Epiphytic microbiota emits compounds that characterize the smell of leaves, flowers, and fruits (Peñuelas *et al.* 2014; Sangiorgio *et al.* 2022), and the quantity of flowers combined with the offered resource and microbial volatiles in attracting pollinators is an essential factor to consider. Many of the smells that guide these insects are produced by epiphytic microorganisms; therefore, it is necessary to analyze these interaction dynamics that are useful in the management of cultivated plants, considering that modifications in the profile of emitted compounds can interfere with the behavior of these insects.

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REFERENCES

ACOSTA, M.; ALVARADO, Y.; CRUZ, M.; LEIVA, M.; DELGADO, L. 2005. Micobiota epífita y contaminantes fungosos del establecimiento in vitro de *Eucalyptus grandis*. Manejo Integrado de Plagas y Agroecología. 75:60-63.

AGRIOS, G.N. 2005. Plant pathology. Fifth edition. Elsevier Academic Press. EE. UU. 922p.

AIZEN, M.A.; VÁZQUEZ, D.P.; SMITH-RAMÍREZ, C. 2002. Historia natural y conservación de los mutualismos planta-animal del bosque templado de Sudamérica austral. Revista chilena de historia natural. 75(1):79-97. http://dx.doi.org/10.4067/S0716-078X2002000100008

ÁLVAREZ, L.J.; LUCIA, M.; RAMELLO, P.J.; PINO, M.D.; ABRAHAMOVICH, A.H. 2014. Abejas asociadas a cultivos de berenjena (*Solanum melongena* L., Solanaceae) en invernadero del Cinturón Hortícola de La Plata, Buenos Aires, Argentina. Revista de la Facultad de Agronomía. 113(2):211-217.

ASOCIACIÓN ESPAÑOLA DE ENTOMOLOGÍA; JARDÍN BOTÁNICO ATLÁNTICO; CENTRO IBEROAMERICANO DE LA BIODIVERSIDAD. 2002. Polinizadores y biodiversidad. Apolo Observatorio de Agentes Polinizadores. España. 160p. Available from Internet in: https://apolo.entomologica.es/cont/materiales/informe_tecnico.pdf

AVARGUÈS-WEBER, FINKE, V.; NAGY, M.; SZABÓ, T.; D'AMARO, D.; DYER, A.G.; FISER, J. 2020. Different mechanisms underlie implicit visual statistical learning in honey bees and humans. Proceedings of the National Academy of Sciences. 117(41):25923-25934. https://doi.org/10.1073/pnas.1919387117

CALÍN-SÁNCHEZ, Á.; LECH, K.; SZUMNY, A.; FIGIEL, A.; CARBONELL-BARRACHINA, Á.A. 2012. Volatile composition of sweet basil essential oil (*Ocimum basilicum* L.) as affected by drying method. Food Research International. 48(1):217-225. https://doi.org/10.1016/j.foodres.2012.03.015

CAÑEDO, T.; AMES, T. 2004. Manual de laboratorio para el manejo de hongos entomopatógenos. International Potato Center. 62p.

CANO, M.A. 2011. Interacción de microorganismos benéficos en plantas: micorrizas, *Trichoderma* spp. y *Pseudomonas* spp. Una revisión. Revista U.D.C.A Actualidad & Divulgación Científica. 14(2):15-31. https://doi.org/10.31910/rudca.v14.n2.2011.771

CARMONA-DÍAZ, G.; GARCÍA-FRANCO, J.G.; HERNÁNDEZ CARMONA, S.; RETURETA APONTE. A.; QUINTANA MORALES, P.C.; LÓPEZ GALINDO, A.; GARCÍA ORDUÑA, F. 2017. Forrajeo y polinización del arbusto *Malpighia* glabra (Malpighiaceae) y la orquídea Oncidium *cosymbephorum* (Orchidaceae) por abejas *Centris* (Apidae) en la isla Agaltepec, Veracruz, México. eNeurobiología. 8(19):181217.

CARVALHO, A.M.; BEGO, L.R. 1995. Seasonality of dominant species of bees in the Panga Ecological Reserve, Cerrado, Uberlândia, MG. Anais da Sociedade Entomológica do Brasil. 24(2):329-337. https://doi.org/10.37486/0301-8059.v24i2.1034

CASTRO-RESTREPO, D.; DOMINGUEZ, M.I.; GAVIRIA-GUTIÉRREZ, B.; OSORIO, E.; SIERRA, K. 2022. Biotization of endophytes *Trichoderma asperellum* and *Bacillus subtilis* in Mentha *spicata microplants* to promote growth, pathogen tolerance and specialized plant metabolites. Plants. 11(11):1474. https://doi.org/10.3390/plants11111474

CHALCHAT, J.C.; ÖZCAN, M.M. 2008. Comparative essential oil composition of flowers, leaves and stems of basil (*Ocimum basilicum* L.) used as herb. Food Chemistry. 110(2):501-503. https://doi.org/10.1016/j.foodchem.2008.02.018

CLARKE, S.C. 2018. Collins and Lyne's Microbiological Methods. British Journal of Biomedical Science. 61(2):113. https://doi.org/10.1080/09674845.2004.11978056

CRAWLEY, M.J. 2012. The R book. John Wiley & Sons. 1051p. https://doi.org/10.1002/9781118448908

DAGENAIS, T.R.; KELLER, N.P. 2009. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. Clinical Microbiology Reviews. 22(3):447-465. https://doi.org/10.1128/CMR.00055-08

DAVIS, T.S.; BOUNDY-MILLS, K.; LANDOLT, P.J. 2012. Volatile emissions from an epiphytic fungus are semiochemicals for eusocial wasps. Microbial Ecology. 64(4):1056-1063. https://doi.org/10.1007/s00248-012-0074-2

DE BRUYNE, M.; BAKER, T.C. 2008. Odor detection in insects: volatile codes. Journal of Chemical Ecology. 34(7):882-897. https://doi.org/10.1007/s10886-008-9485-4

DICKSCHAT, J.S.; CELIK, E.; BROCK, N.L. 2018. Volatiles from three genome sequenced fungi from the genus *Aspergillus*. Beilstein Journal of Organic Chemistry. 14(1):900-910. https://doi.org/10.3762/bjoc.14.77

FOLEY, K.; FAZIO, G.; JENSEN, A.B.; HUGHES, W.O. 2014. The distribution of *Aspergillus* spp. opportunistic parasites in hives and their pathogenicity to honeybees. Veterinary Microbiology. 169(3-4):203-210. https://doi.org/10.1016/j.vetmic.2013.11.029

GARCÍA-CONDE, K.B.; CERNA-CHÁVEZ, E.; OCHOA-FUENTES, Y.M.; VELÁZQUEZ-GUERRERO, J.J. 2024. *Aspergillus* oryzae: Una oportunidad para la agricultura. Revista mexicana de fitopatología. 42(1). https://doi.org/10.18781/r.mex.fit.2302-2

GARCÍA-GARCÍA, M.C.; ORTIZ, P.L.; DÍEZ DAPENA, M.J. 2001. Pollen collecting behaviour of *Apis mellifera* during one day. Grana. 40(4-5):205-209. https://doi.org/10.1080/001731301317223141

GERMAINE, K.; KEOGH, E.; GARCIA-CABELLOS, G.; BORREMANS, B.; LELIE, D.; BARAC, T.; OEYEN, L.; VANGRONSVELD, J.; MOORE, F.P.; MOORE, E.R.B.; CAMPBELL, C.D.; RYAN, D.; DOWLING, D.N. 2004. Colonisation of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiology Ecology. 48(1):109-118. https://doi.org/10.1016/j.femsec.2003.12.009

GIURFA, M. 2013. Cognition with few neurons: higher-order learning in insects. Trends in Neurosciences. 36(5):285-294. https://doi.org/10.1016/j.tins.2012.12.011

GIURFA, M.; EICHMANN, B.; MENZEL, R. 1996. Symmetry perception in an insect. Nature. 382(6590):458-461. https://doi.org/10.1038/382458a0

GONZÁLEZ-ZÚÑIGA, J.A.; GONZÁLEZ-SÁNCHEZ, H.M.; GONZÁLEZ-PALOMARES, S.; ROSALES-REYES, T.; ANDRADE-GONZÁLEZ, I. 2011. Microextracción en fase sólida de compuestos volátiles en albahaca (*Ocimum basilicum* L.). Acta Universitaria. 21(1):17-22. https://doi.org/10.15174/au.2011.48

GRANADOS-SÁNCHEZ, D.; LÓPEZ-RÍOS, G.F.; HERNÁNDEZ-GARCÍA, M.Á.; SÁNCHEZ-GONZÁLEZ, A. 2003. Ecología de las plantas epífitas. Revista Chapingo. Serie Ciencias Forestales y del Ambiente. 9(2):101-111.

HALLMANN, J.; QUADT-HALLMANN, A.; MAHAFFEE, W.F.; KLOEPPER, J.W. 1997. Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology. 43(10):895-914. https://doi.org/10.1139/m97-131

HEMPEL DE IBARRA, N.; HOLTZE, S.; BÄUCKER, C.; SPRAU, P.; VOROBYEV, M. 2022. The role of colour patterns for the recognition of flowers by bees. Philosophical Transactions of the Royal Society. 377(1862):20210284. http://doi.org/10.1098/rstb.2021.0284

HÜE, T.; CAUQUIL, L.; FOKOU, J.H.; DONGMO, P.J.; BAKARNGA-VIA, I.; MENUT, C. 2015. Acaricidal activity of five essential oils of Ocimum species on *Rhipicephalus* (*Boophilus*) *microplus* larvae. Parasitology Research. https://doi.org/10.1007/s00436-014-4164-6

HUNG, K.L.J.; KINGSTON, J.M.; ALBRECHT, M.; HOLWAY, D.A.; KOHN, J.R. 2018. The worldwide importance of honeybees as pollinators in natural habitats. Proceedings of the Royal Society B: Biological Sciences. 285(1870):20172140. http://doi.org/10.1098/rspb.2017.2140

JIANG, Y.; YE, J.; LI, S.; NIINEMETS, Ü. 2016. Regulation of floral terpenoid emission and biosynthesis in sweet basil (*Ocimum basilicum*). Journal of Plant Growth Regulation. 35:921-935. https://doi.org/10.1007/s00344-016-9591-4

KLEIN, A.M.; VAISSIERE, B.E.; CANE, J.H.; STEFFAN-DEWENTER, I.; CUNNINGHAM, S.A.; KREMEN, C.; TSCHARNTKE, T. 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society B: Biological Sciences. 274(1608):303-313. https://doi.org/10.1098/rspb.2006.3721

KONEMAN, A.; DOWELL, S. 1989. Diagnóstico microbiológico. Texto y atlas color. 2ª ed. Ed. Panamericana (México).

LOPES DE CARVALHO, C.A.; MARCHINI, L.C. 1999. Tipos polínicos coletados por *Nannotrigona testaceicornis* e *Tetragonisca angustula* (Hymenoptera, Apidae, Meliponinae). Scientia Agricola. 56:717-722. https://doi.org/10.1590/S0103-90161999000300029

LÓPEZ-ÁVILA, A.; RINCÓN, D.F. 2006. Diseño de un olfatómetro de flujo de aire para medir respuestas olfativas de insectos de tamaño mediano y pequeño. Ciencia y Tecnología Agropecuaria. 7(1):61-65. https://doi.org/10.21930/rcta.vol7_num1_art:61

MATU, F.K.; MURUNGI, L.K.; MOHAMED, S.; DELETRE, E. 2021. Behavioral response of the greenhouse whitefly (*Trialeurodes vaporariorum*) to plant volatiles of *Ocimum basilicum* and *Tagetes minuta*. Chemoecology. 31(1):47-62. https://doi.org/10.1007/s00049-020-00327-z

MENZEL, R. 2001. Behavioral and neural mechanisms of learning and memory as determinants of flower constancy. In: Chittka, L.; Thomson, J.D. (eds). Cognitive ecology of pollination. Ed. Cambridge University Press. p.21-40. https://doi.org/10.1017/CBO9780511542268.003

MICHENER, C.D. 2007. The bees of the world. second edition. Johns Hopkins University Press. EE.UU. 992p.

MORÉ, E.; FANLO, M.; MELERO, R.; CRISTÓBAL, R. 2010. Guía para la producción sostenible de plantas aromáticas y medicinales. Centre Tecnològic Forestal de Catalunya. 265p. Available from Internet in: https://apsb.ctfc.cat/docs/GUIA%20PAM-CASTELLAfinal.pdf

MURILLO PEREA, E.; VIÑA PATIÑO, A. 1999. Determinación de los constituyentes volátiles de la albahaca (*Ocimum* spp.) mediante dos métodos de extracción. Revista Colombiana de Química. 28(1):65-74.

MURILLO, E.; FERNÁNDEZ, K.; SIERRA, D.; VIÑA, A. 2004. Caracterización fisicoquímica del aceite esencial de albahaca. II. Revista Colombiana de Química. 33(2):139-148.

OCAÑA-CABRERA, J.S.; LIRIA, J.; VIZUETE, K.; CHOLOTA-IZA, C.; ESPINOZA-ZURITA, F.; SAEGERMAN, C.; MARTIN-SOLANO, S.; RON-ROMÁN, J. 2022. Pollen preferences of stingless bees in the Amazon region and southern highlands of Ecuador by scanning electron microscopy and morphometry. PLOS One. 17(9):e0272580. https://doi.org/10.1371/journal.pone.0272580

OLLERTON, J. 2021. Pollinators and pollination: nature and society. Pelagic Publishing Ltd. Reino Unido. 336p.

PARRA, G.N. 2001.Guía para la cría y manejo de la abeja Angelita o Virginita *Tetragonisca angustula* Illiger. Serie Ciencia y tecnología. No. 84. Convenio Andrés Bello. Colombia. 43p.

PEÑUELAS, J.; FARRÉ-ARMENGOL, G.; LLUSIA, J.; GARGALLO-GARRIGA, A.; RICO, L.; SARDANS, J.; TERRADAS, J.; FILELLA, I. 2014. Removal of floral microbiota reduces floral terpene emissions. Scientific Reports. 4(1):6727. https://doi.org/10.1038/srep06727

PHOTITA, W.; LUMYONG, S.; LUMYONG, P.; MCKENZIE, E.H.C.; HYDE, K.D. 2004. Are some endophytes of Musa acuminata latent pathogens? Fungal Diversity. 16:131-140.

R DEVELOPMENT CORE TEAM. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing (Austria). Available from Internet in: https://www.R-project.org/

ROSENBLUETH, M.; MARTÍNEZ-ROMERO, E. 2006. Endófitos bacterianos y sus interacciones con los huéspedes. Interacciones moleculares planta-microbio. 19(8):827-837. https://doi.org/10.1094/MPMI-19-0827

SANGIORGIO, D.; CELLINI, A.; SPINELLI, F.; PASTORE, C.; FARNETI, B.; SAVIOLI, S.; RODRIGUEZ-ESTRADA, M.T.; DONATI, I. 2022. Contribution of fruit microbiome to raspberry volatile organic compounds emission. Postharvest Biology and Technology (Países Bajos). 183:111742. https://doi.org/10.1016/j.postharvbio.2021.111742

SILVA LIMA, A.; MILHOMEM, M.N.; SANTOS MONTEIRO, O.; PEREIRA ARRUDA, A.C.; MARTINS DE CASTRO, J.A.; LOPES FERNANDES, Y.M.; SOARES MAIA, J.G.; COSTA-JUNIOR, L.M. 2018. Seasonal analysis and acaricidal activity of the thymol-type essential oil of *Ocimum gratissimum* and its major constituents against *Rhipicephalus microplus* (Acari: Ixodidae). Parasitology Research. 117:59-65. https://doi.org/10.1007/s00436-017-5662-0

SLAA, E.J.; SÁNCHEZ CHAVES, L.A.; MALAGODI-BRAGA, K.S.; HOFSTEDE, F.E. 2006. Stingless bees in applied pollination: Practice and perspectives. EDP Sciences Apidologie. 37(2):293-315. https://doi.org/10.1051/apido:2006022

SRINIVASAN, M.V.; ZHANG, S.W.; WITNEY, K. 1994. Visual discrimination of pattern orientation by honeybees: performance and implications for ‘cortical’ processing. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. 343(1304):199-210. https://doi.org/10.1098/rstb.1994.0021

SRINIVASAN, M.V.; ZHANG, S.W.; ZHU, H. 1998. Honeybees link sights to smells. Nature. 396(6712):637-638. https://doi.org/10.1038/25272

STEINER, S.; ERDMANN, D.; STEIDLE, J.L.; RUTHER, J. 2007. Host habitat assessment by a parasitoid using fungal volatiles. Frontiers in Zoology. 4:3. https://doi.org/10.1186/1742-9994-4-3

STONE, J.K.; POLISHOOK, J.D.; WHITE, J.F. 2004. Endophytic fungi. In: Mueller, G. M.; Bills, G. F.; Foster, M. S. (eds). Biodiversity of fungi: inventory and monitoring methods. Elsevier Academic Press. EEUU. p.241-270.

SUNESSON, A.L.; VAES, W.; NILSSON, C.; BLOMQUIST, G.; ANDERSSON, B.; CARLSON, R. 1995. Identification of volatile metabolites from five fungal species cultivated on two media. Applied and Environmental Microbiology. 61(8):2911-2918. https://doi.org/10.1128/aem.61.8.2911-2918.1995.

TORRETTA, J.P.; MEDAN, D.; ROIG ALSINA, A.; MONTALDO, N.H. 2010. Visitantes florales diurnos del girasol (*Helianthus annuus*, Asterales: Asteraceae) en la Argentina. Revista de la Sociedad Entomológica Argentina. 69(1-2):17-32.

TSAVKELOVA, E.A.; CHERDYNTSEVA, T.A.; BOTINA, S.G.; NETRUSOV, A.I. 2007. Bacteria associated with orchid roots and microbial production of auxin. Microbiological Research. 162(1):69-76. https://doi.org/10.1016/j.micres.2006.07.014

VANNETTE, R.L. 2020. The floral microbiome: plant, pollinator, and microbial perspectives. Annual Review of Ecology, Evolution, and Systematics. 51(1):363-386. https://doi.org/10.1146/annurev-ecolsys-011720-013401

VÉLEZ, P.E.; POSADA, F.J.; MARÍN, P.; GONZALEZ, M.T.; OSORIO, E.; BUSTILLO, A.E. 1997. Técnicas para el control de calidad de formulaciones de hongos entomopatógenos. Cenicafé. 24p.

WOODCOCK, T.S.; LARSON, B.M.H.; KEVAN, P.G.; INOUYE, D.W.; LUNAU, K. 2014. Flies and flowers II: floral attractants and rewards. Journal of Pollination Ecology. 12:63-94. https://doi.org/10.26786/1920-7603(2014)5