



Full Length Article

Ability of Honey and Aqueous Plant Extracts on the Attraction of Bees during Flowering Period in Mango (*Mangifera indica*) var. Ataulfo Cultivation

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Abstract

In recent decades there have been low yields of mango (*Mangifera indica* L.) var. Ataulfo, related to the presence of pests and diseases, for which the irrational use of pesticides has increased, causing a deficit of pollinators. Among the alternatives allowing attracting pollinators are plant extracts. The study was carried out in the mango orchard var. Ataulfo in the Soconusco region, Chiapas, México in the years 2020 and 2021. Six treatments were used: 1) 1.25% aqueous skunk plant extract, 2) 1.25% aqueous skunk plant extract + 0.5% *Melipona solani* honey, 3) 0.5% *M. solani* honey, 4) 2% commercial garlic extract, 5) 2% commercial clove extract and 6) water as control. For each of the treatments, five selected trees were sprayed one day before the application of the treatments and one day after the bees attracted with entomological nets were collected during the following six days, which were taxonomically identified. The results showed that the most attractive treatments were skunk extract 1.25% + *M. solani* honey 0.5% and *M. solani* honey (0.5%), with highly significant differences ($p < 0.001$). The days of greatest attraction were the second and fourth days after treatment application. The use of *M. solani* honey + skunk extract has potential as a pollinator attractant in mango var. Ataulfo in the Soconusco region, Chiapas. © 2023 Friends Science Publishers

Keywords: Attractants; Pollinators; Extracts; Honey; Stingless bee

Introduction

The attraction of insects to plant flowers is related to the presence of volatile organic compounds (VOCs), as well as visual clues, which are key to their pollination (Ramírez and Davenport 2016; Bohman *et al.* 2020). About 75% of the 111 main agricultural crops depends on insects to have a higher production and quality of the fruits, for which it is necessary to have integrated strategies that allow the pollination of the crops and ensure an economically sustainable production (Isaacs *et al.* 2017). In this sense, the cultivation of mango (*Mangifera indica* L.) is considered worldwide of third in importance due to its high levels of production and export (Igbari *et al.* 2019). The cross-pollination carried out by insects ensures its production, with bees and flies being the main contributors (Fajardo *et al.* 2008).

Mexico is the fifth mango-producing country in the world and the second exporter with 19.6%. Chiapas ranks

fourth in production, contributing 14% of national production (SAGARPA 2019). In the Soconusco area, Chiapas, mango cultivation faces different problems due to the presence of pests and diseases, which has led producers to apply large amounts of pesticides, thus causing a decrease in the presence of beneficial insects. This is causing a deficit of pollinators, negative effects on the health of living beings and the environment (Novais *et al.* 2016; De Oliveira *et al.* 2019).

Likewise, a decrease in the yield of annual mango production has been reported. In 2015 the yield was $7.12 \text{ t} \cdot \text{ha}^{-1}$, while, in 2019, it was $6.49 \text{ t} \cdot \text{ha}^{-1}$ (SAGARPA 2019), which is associated with the decrease in production yield in mango with pollinator deficiency. Among the environmentally sustainable alternatives that minimize the use of pesticides and attract pollinators, are plant extracts, which contain secondary metabolites and VOCs, such as: saponins, tannins, alkaloids, aliphatic compounds, fatty acid derivatives, terpenes, among others (Ignat *et al.* 2011; Stitz *et al.* 2014;

Vera-Delgado *et al.* 2016). There are antecedents of the use of plant extracts and derivatives of bee products in which they were observed to have the capacity to act as attractants of pollinating insects, control against pests and diseases and influence on floral induction, (Escobedo-Mendoza 2021).

Some research papers mention some VOCs present in plants such as linalool, limonene, 2-phenylethyl propionate, eugenol and geraniol with the ability to attract bees as pollinators (Jayaramappa *et al.* 2011; Pashte *et al.* 2015). Compounds such as; nerolidol, dimethylsulfide, diethylsulfides, di-n-propylsulfide, benzyl polysulfides, triterpenes, coumarins and flavonoids (Luz *et al.* 2016; Alves *et al.* 2019), which are related to the attraction of bees. Plant extracts of *Petiveria alliacea* L. have been applied to control diseases and flower inducers in mango, while an increase in the number of insects visiting the flowers has been observed (Escobedo-Mendoza 2021). The above described points lead to the fact that honey and plant extracts from the *P. alliacea* L. plant are an alternative to increase the visits of pollinating insects such as bees. Therefore, the objective of the work was to evaluate the capacity of *M. solani* honey and the aqueous plant extract of *P. alliacea* L. to attract bees during the flowering period in mango (*M. indica* L) var. Ataulfo.

Materials and Methods

Experimental treatments

The treatments were established in the mango orchard of Rancho "San Juan" (Fig. 1), Tapachula, Chiapas (orchard with conventional management) 14.5453 N, - 92.1756 W, where the plantation age ranges from 12 to 15 years. in production. Six treatments were used; 1) 1.25% aqueous skunk plant extract, 2) 1.25% aqueous skunk plant extract + 0.5% *M. solani* honey, 3) 0.5% *M. solani* honey, 4) 2% commercial garlic extract, 5) 2% commercial clove extract and 6) water as control.

Preparation of aqueous plant extracts

For the preparation of the extracts, virgin honey from the *M. solani* stingless bee and leaves of skunk plants (*P. alliacea*) were used. Virgin honey was obtained from the Asociación de Meliponicultores del Soconusco, Chiapas, and stored at room temperature; skunk leaves were obtained from Rancho "Los Gallardos", 14.865486 N, -92.191845 W, at 320 m s. no. m in Tuxtla Chico, Chiapas.

Fresh skunk leaves were collected in the morning (07:00 h), stored in properly labeled hermetically sealed bags, and later transferred to the ECOSUR Toxicology laboratory, Tapachula unit, for later use. The skunk leaves were disinfected with 1% sodium hypochlorite and washed with water and allowed to dry. A 500 g dried material was weighed, then they were cut into small squares of approximately one centimeter, and placed in 2 L of water at 95°C at a dilution (1:4 w/v) and left to rest for 24 h in the

dark. After this, the extracts were filtered, kept in the dark until use. Distilled water was used for honey dilution (Escobedo-Mendoza 2021).

In addition to the above, the commercial garlic (*Allium sativum* L.) extract (garlic plus) was purchased from the naturist distributor MAYAMEX S.A. de C.V. Also the clove (*Syzygium aromaticum* L.) Merr. & L.M. Perry) hydroglycolic extract was obtained from the distributor Angel de Oro.

Experimental design

Five trees per treatment were used in a completely randomized design, to which two applications were made in two different mango flowering periods (1st. late November 2020 and 2nd. early January 2021) called evaluation periods. The sprays were applied using backpack sprayers with a capacity of 20 L throughout the treetops. The field dilutions of the different treatments were carried out with deep well water, according to the inflorescences they presented, 4 L of deep well water was used per tree.

The collection of bees was carried out with aerial entomological nets by beating (Ramírez *et al.* 2014), in the evaluation periods, which were carried out in the treetop area. The collection began one day before the application and up to six days after having applied the treatments, at the following times: 08 to 11, 11 to 14 and 14 to 17 h. The captured bees were mounted on entomological pins and stored in an entomological box. The taxonomic identification was carried out with the group of researchers from the ECOSUR Bee Team, San Cristóbal de las Casas unit, Chiapas. Data on climatic conditions of temperature, relative humidity and dew point were obtained, provided by CONAGUA.

Abundance index

After the taxonomic identification of the bees was found and using only the identified species, the abundance index value per treatment was determined. The value was determined by the following equation:

$$IA = \frac{n}{(N-1)} \times T$$

Where IA= abundance index, n= observed level, N= number of levels found and T= total number of bees.

Statistical analysis of data

The data collected from all the exposed variables were analyzed using an ANOVA and a comparison of means by Tukey $\alpha = 0.05$. For the climatic variables, a Spearman correlation test was performed for non-parametric data, using the Infostat 2018 statistical program with conversion to R, both data were analyzed with 99% confidence. To obtain the Shannon (H) and Simpson (ID) diversity indices of the different bee species, the statistical program RStudio version 1.4.1106 was used.

Results

Attraction of bees to different treatments of both applications

A total of 298 specimens were collected, with ten bee species of bees in both evaluation periods; in the first period 86 individuals were collected and in the second period 212 individuals (Table 1). Highly significant differences ($p < 0.0001$) were observed in relation to the evaluation periods and the treatments applied. Treatments 2 (1.25% skunk plant extract + 0.5% honey) and 3 (0.5% honey) in both periods, registered a greater attraction of bees. Regarding the richness and diversity of species in both periods of application, treatment 3 obtained the greatest diversity of bees, on contrary, the treatment of skunk 1.25% and treatment 6 of water (control) the values were marginal (Table 2).

Regarding the rounding periods and the evaluation periods, highly significant differences were observed ($P < 0.0001$), registering a greater number of bees collected in the second period that was from January 2 to 9, 2021. On the contrary, in the first evaluation period, the treatment with the greatest richness and diversity was treatment 6 (control), and the richness and diversity of species (H'), was higher in the second evaluation period. Treatments 2 (1.25% skunk plant extract + 0.5% honey) and 3 (0.5% honey) of the second evaluation period were the ones with the greatest diversity and richness, regardless of the number of bees that visited the treated plants. The diversity Shannon index, both in the first and second rounding periods, marginal data were obtained with values less than two (Fig. 1).

Diversity and richness in the two evaluation periods

In the two evaluation periods, days two and four after having applied the treatments, stand out with diversity values of 0.65 and richness of 2 (Fig. 2–3). For the climatic conditions, Spearman's correlation tests were applied to find out if there was a relationship between the presence of bees and the climatic conditions that were taken into account for the study, all the climatic variables had a positive correlation, but it was found that both the temperature (0.91) and dew point (0.73), have a high correlation with the presence of bees, comparison of means by Tukey $\alpha = 0.05$.

Abundance index of two evaluation periods

Highly significant ($P < 0.0001$) differences were found between both the periods and treatments. It was observed that the second period was the one with the highest abundance of species. Finding that in the first period the similar species and with the highest AI value were *Apis mellifera* and *Trigona nigerrima*, while both these same species differed in the second period with a minimal difference, being the most abundant *A. mellifera*.

Table 1: Bee species and number collected during the first and second evaluation period in San Juan mango orchard

Bee species	No. of bees collected		
	First period	Second period	Group periods
<i>Apis mellifera</i>	3	88	91
<i>Auguchlora sp</i>	1	0	1
<i>Caenagochlora inermis</i>	0	1	1
<i>Dialictus</i>	0	1	1
<i>Nannotrigona perilampoides</i>	0	1	1
<i>Oxytrigona mediorufa</i>	1	0	1
<i>Tetragonisca Angustula</i>	0	6	6
<i>Trigona fulviventris</i>	4	41	45
<i>Trigona fuscipennis</i>	63	30	93
<i>Trigona nigerrima</i>	14	44	58
Total	86	212	298

Table 2: Evaluation of periods with the different bee attractant treatments

Period of evaluation	Net time	Average \pm SE
2	14:00–16:25	2.33 \pm 0.02 A
2	08:00–10:25	2.3 0.0 \pm 2 A
2	11:00–13:25	2.3 \pm 0.02 A
1	11:00–13:25	2.17 \pm 0.02 B
1	08:00–10:25	2.17 \pm 0.02 B
1	14:00–16:25	2.13 \pm 0.02 B

*same letters showed no significant differences $P < 0.0001$

T1) 1.25% aqueous skunk plant extract, T2) 1.25% aqueous skunk plant extract + 0.5% *M. solani* honey, T3) 0.5% *M. solani* honey, T4) 2% commercial garlic extract, T5) 2% commercial clove extract and T6) water as control

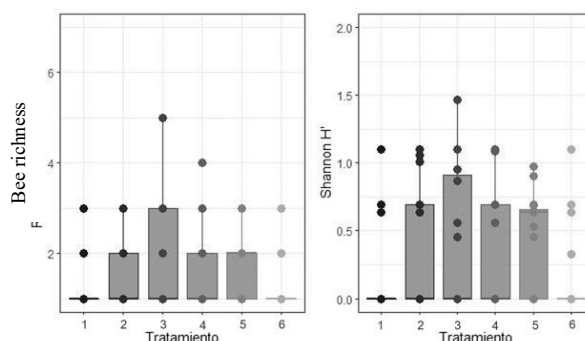


Fig. 1: Bee diversity and richness for the first and second treatment application in the San Juan mango orchard

Discussion

In this study, treatments 2 and 3 registered a greater attraction in both periods, which is related to the VOCs that are reported in the skunk plant (diethylsulfides, di-n-propylsulfide, terpene derivatives: α and β -pinenes and limonene) (1p: 0.32 and 0.33, respectively, in 2p: 0.76 and 0.72, respectively), which can be considered as potential bee attractants (Feinstein *et al.* 2008; Luz *et al.* 2016; Alves *et al.* 2019; Cantú-Ayala *et al.* 2019).

In the first period, the number of bees that visited the trees with the applied treatments was lower (Minimum 0.0 and maximum 4.0, in general), which could be attributed to

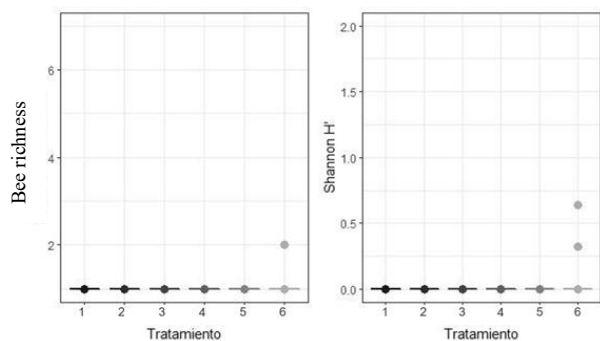


Fig. 2: Bee diversity and richness during the first evaluation period

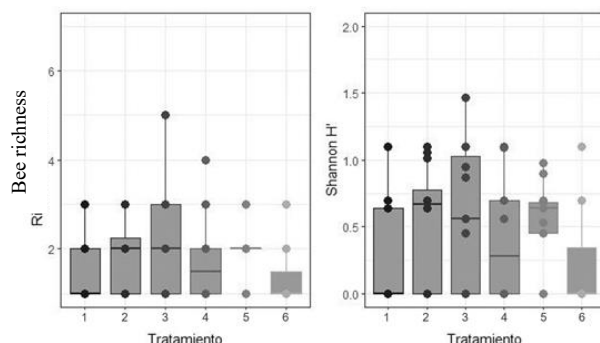


Fig. 3: Bee diversity and richness during the first evaluation period

the fact that it is the first flowering and there are generally few flowers in the orchard. However, in the second period (more intense) a greater number of bees was recorded as floral visitors (524 bees). Likewise, it is necessary to consider what González-Céspedes *et al.* (2019) indicate that the most important climatic parameters on the activity of bee visits to floral resources were temperature and dew point, which agrees with these findings.

In this study, honey from *M. solani* was used for the first time as part of bee attractants in crops of interest (0.01% v/v). In contrast, *A. mellifera* honey has been used as an attractant at population considered low, between 1 and 2% (Pashte *et al.* 2015; Kumari and Rana 2018; Wankhede *et al.* 2018). The results obtained showed that *M. solani* honey had the ability to attract bees at even lower concentrations (0.5%).

Regarding diversity and richness of species in the first period, treatment 6 (control) was the one that registered the highest number (Shannon H: 1.2), which can be attributed to the fact that in this treatment there was the presence of young mango leaves, which is used by some bees of the genus *Trigona*. In this context Camacho-Vargas (1966), Araúz *et al.* (2013); Cruz *et al.* (2016) analyzed that the bees eat the edges of the new leaves of the plant. Also, when the leaves are tender, the bees eat the entire sheet and also the succulent parts of the buds. These bees use materials such as resins and fibers that they take from the leaves and bark of certain plants to build their nests. The bees causing this problem to belong to the *Trigona* genus, Meliponini tribe, Apinae subfamily,

with *T. fuscipennis* being identified within these species (López-Guillen *et al.* 2019).

At any time of the day there was an increase in the number of bees that were collected, with a decline in the last hours of rounding up (4:00 p.m.) onwards (0.00). On days 2 to 4, a greater presence of bees was found, which agrees with Kumari and Rana (2018) in which they applied plant extracts and honey as an attractant in the sesame crop and observed the maximum number of bees from the second to the fourth day as floral visitors. The species that predominated in this study was *A. mellifera* both in the first evaluation period and in the second, which benefits mango cultivation since De la Peña *et al.* (2018) related higher pollination percentages and greater floral mooring with the ecosystem service provided by this species.

The distance may be related to the scarce presence of *T. angustula*, due to the fact that the meliponary that the San Juan mango orchard has is located at a distance of approximately 1 km and searches for bee nests were carried out in advance without stinger near the experimental plot, but they were not found. In stingless bees, the foraging radius was 0.5 km (Sánchez *et al.* 2008) and this may be the reason why the visit of this species was not the best. According to Heard (1999) *T. angustula* is another bee species that pollinates mango crops. In subsequent research work, the identification of VOCs that could be associated with the attraction of bees as pollinators will be carried out. Finally, it is important to highlight that there is still a lack of research to be carried out and to develop alternatives that improve the productivity of the Ataulfo variety control associated with pollination.

Conclusion

M. solani honey and skunk (*P. alliacea* L.) plant extract had significant capacity to attract bees to flowering mango in Soconusco, Chiapas. The climatic conditions are extremely important in the behavior of the bees, since any change in the temperature and the dew point can affect them and, consequently, the visit of the bees to the inflorescences of the trees. It was found that the days of greatest attraction occurred from 2 to 4 days after the application of the treatments.

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Author Contributions

JGC conceived the idea, directed the investigation and contributed to the writing of manuscript; AGP conducted the fieldwork, analyzed the data, and worked on the manuscript; LCL contributed to the manuscript and to the methodology of the chemical area of the work; JALG contributed to the

idea of the work and to the design, contributed to the manuscript from start to finish; JAMR contributed to the identification of the bee species, as well as to the improvement of the final manuscript; RTS contributed to revise the manuscript, to revise the English and support in the analysis of the data; VJAF contributed to the idea of the work and to the design, contributed to the manuscript from start to finish and to the analysis of the data.

Conflict of Interest

All authors have no conflict of interest about the results presented along this manuscript.

Data Availability

If it is necessary all authors could trust you all the data obtained during this research.

Ethics Approval

All experiments were performed with the ethics approval for bee collection.

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