

RESPONSE OF Bemisia tabaci Genn TO THE ASSOCIATION TOMATO-AROMATIC PLANT

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ABSTRACT

The silverleaf whitefly (Bemisia tabaci) is the most harmful pest for vegetables in tropical zones. One alternative for its ecological management is the use of repellent aromatic plants. The hypothesis of this study is that the aromatic plants that diminish the attraction of B. tabaci to tomato have the capacity to prevent the infestation of this pest in the tomato crop when an intercropping system of tomato-aromatic plant is established. The objective of this study was to evaluate by means of the technique of olfactometry, the response of B. tabaci to the volatiles emitted by the crushed leaves of tomato and by the combination tomato + aromatic plant. The aromatic plants with highest activity in the olfactometry tests were intercropped in a tomato crop in the field. In the bioassays of olfactometry, a significant decrease was observed in the attraction of B. tabaci to crushed leaves of tomato + some aromatic plants compared with the attraction to tomato alone. The repellence indices (IR) showed that the aromatics Lavandula angustifolia Mill. (IR, 0.1), Petiveria alliacea L. (IR, 0.48), Petroselinum crispum Mill. (IR, 0.28) and Thymus vulgaris (IR, 0.5) had the greatest effect as repellent of adults. By integrating these aromatic species through intercropping in the tomato field crop, no significant difference was observed in the population density of eggs and adults of B. tabaci in the tomato foliage in regard to what was observed in the control (only tomato). The incidence and severity of viral symptoms did not decrease from the presence of the aromatic species intercropped in the tomato crop.

Keywords: insect repellence, associated crop, tomato pests, biorational management.

One of the limitations in tomato production is the damage caused by the whitefly *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae), which not only causes damage when it feeds on the sap of the plants, but also efficiently transmits more than 100 viruses of the begomovirus group (Khatun *et al.*, 2020). This pest is distributed worldwide and is distinguished by a wide range of hosts, high fertility, and high capacity for virus transmission (Islam *et al.*, 2018). Furthermore, *B. tabaci* has potential for developing resistance to chemical insecticides (Grávalos *et al.*, 2015; Rosen *et al.*, 2015.

INTRODUCTION



Editor in Chief: Dr. Fernando C. Gómez Merino

Received: June 23, 2021. Approved: May 02, 2022. **Published in Agrociencia:** June 17, 2022.

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In the interaction *B. tabaci* – host plant, the visual attraction and aroma of the plants is determinant in the infestation process (Tsueda *et al.*, 2014). On the other hand, it is also known that the release of volatiles of some plant species interferes with the attraction and establishment of *B. tabaci* in host plants (Tosh and Brogan, 2015). Some aromatic species have been successfully used as pest repellent plants. These plants generally emit a set of volatile organic compounds (VOC) with adverse effects against the phytophagous insects; which include repellence, anti-alimentary activity, growth retardation and lower fertility of the pest insects (Cook *et al.*, 2007; Sujayanand *et al.*, 2015).

The aromatic plants intercropped with the crop of interest can affect the capacity of the pest insects to discriminate between the volatiles of the host plants and those of the aromatic plants, which affects the establishment and colonization of the phytophage (Cook *et al.*, 2007). In specific studies on the management of *B. tabaci* in vegetables by means of intercropping aromatic plants, a reduction has been observed in the population of *B. tabaci*, along with a decrease in incidence and severity of begomovirus and improvement in fruit yields (Sujayanand *et al.*, 2015). For example, the tomato crop intercropped with *Coriandrum sativum* reduced the number of adults of *B. tabaci*, delayed the appearance of the tomato yellow spot virus (ToyMov), diminished the severity of the disease, and improved the yields (Cook *et al.*, 2007).

Carvalho *et al.* (2017) found that the tomato crop intercropped with aromatic plants of *Ocimum basilicum* and *Coriandrum sativum* reduces the incidence of *Bemisia tabaci*. The use of VOC of aromatic plants has even been reported against *B. tabaci* through assays of repellence and dissuasion, such as the use of essential oils of *Litsea cubeba* Lour (Wagan *et al.*, 2017). This suggests that the aromatic plants not only have great potential for direct use, intercropped with the plants of interest, but also as a source of compounds which can be used in the future as plant-based insecticides.

This study was established with the hypothesis that the aromatic plants that diminish the attraction of *B. tabaci* to tomato, have the capacity to prevent the infestation of this pest in the tomato crop when it is established in an intercropped system of tomato-aromatic plant. Therefore, the objective of the study was to evaluate the response of *B. tabaci* to 18 species of aromatic plants through laboratory bioassays by means of an olfactometer, to later evaluate the inclusion of intercropped aromatic plants on the populational suppression of eggs and adults of *B. tabaci* in the tomato crop on the field.

MATERIALS AND METHODS

Study sites and obtainment of aromatic plants

The study of response of *Bemisia tabaci* to aromatic plants by olfactometry was conducted in the laboratory of agricultural pests of the Conkal Technological Institute. The field study where evaluation was done of the effect of aromatic plants intercropped with tomato was established in the community of Chenche del las Torres, in Temax, Yucatan, Mexico (21.3° N, 88.98° W at 12 m altitude).

Plant material for the laboratory tests was obtained from aromatic plants in vegetative development cultivated in the greenhouse in plastic bags of 2 L. The aromatic plants

evaluated (Table 1) were maintained in the greenhouse under natural conditions, with constant irrigation, and did not receive fertilization or application of pesticides.

Evaluation of attraction in olfactometer

The evaluations of attraction were done using samples of crushed foliage (4 g) as aroma source. The foliage was obtained from tomato plants of 40 d after emergence and aromatic plants (AP) in vegetative development. The adults of *B. tabaci* biotype B used in the experiments were obtained from a colony established in the greenhouse in eggplant. Originally the colony was obtained from crops of habanero chili (Ballina-Gómez *et. al.*, 2013).

In order to compare the responses of the adult insects to the tomato and to the combination of tomato + aromatic plants, two assays were established. In the first assay the comparison of attraction was done between 1: humidified air and 2: attraction to only tomato or attraction to the combination tomato + aromatic plant. In the second assay the comparison was done of attraction to only tomato and attraction to the combination tomato + aromatic plant.

The experiments were carried out in an olfactometer of two arms of high-density polyethylene. The sections of the olfactometer have the following dimensions: intermediate chamber, 3 cm height; entrances of air and a stopper, 1.5 cm thickness; circular central orifice, 8 mm at the base of the chamber to introduce the insects to be evaluated (Shao-Jian *et al.*, 2014). The air of entrance to the olfactometer was propelled with a pump and was purified by being passed through a jar which contained activated

Table 1. List of aromatic species studied with their identification codes.

Common name	Scientific name	Code
Thyme	Thymus vulgaris L.	TM
Chives	Allium schoenoprasum L.	CB
Oreganon	Plectranthus amboinicus Lour.	ON
Basil	Ocimum basilicum minimum L.	ALHC
Lavander	Lavandula angustifolia Mill.	LV
Citronella	Pelargonium citrosum Van.	CN
Rosemary	Rosmarinus officinalis L.	RM
Bush basil	Ocimum micranthum Willd.	XK
Epazote	Dysphania ambrosioides L.	EZ
Rue	Ruta graveolens L.	RD
Melissa	Melissa officinalis L.	TR
Coriander	Coriandrum sativum L.	CL
Mexican oregano	Lippia graveolens Kunth.	ORM
Wild skunk	Petiveria alliacea L.	PY
Peppermint	Mentha spicata L.	HB
Neem	Azadirachta indica A. Juss.	NM
Lemon grass	Cymbopongon citratus DC.	ZL
Parsley	Petroselinum crispum Mill.	PJ

carbon and an Erlenmeyer flask filled with water. Prior to the entrance of the air to the spaces containing the aroma sources (glass tubes of 50 mL), a flowmeter was placed to maintain the airflow at 1 L min^{-1} .

In the assays the procedure described by Shao-Jian *et al.* (2014) was followed. The adults of *B. tabaci* were placed in a glass vial of 5 mL in the arm of entrance to the chamber. The air flow was maintained for 5 minutes and then was turned off for 2 min, which permitted the insects to move toward the arm that contained the preferred stimulus (aroma source). Positive response was recorded when the insect moved to one of the arms containing the aroma source. The insects that did not respond were those that remained immobile, or which did not head completely to one of the arms during the 2 min of test. Each assay consisted of the individual evaluation of 20 insects. Each assay was replicated five times (replications) for each aroma source (aromatic plant).

After each assay, the chambers were cleaned allowing air to circulate through the entrance chamber where a piece of cotton impregnated with acetone was placed. After 5 minutes of air circulation, all of the components of the olfactometer where the air circulates in the interior of the equipment were washed with distilled water (Carvalho *et al.*, 2017).

Establishment of the tomato crop in the field

The tomato seedlings of 25 d were transplanted in rows intercropped with plants of each one of the four aromatic species: lavender (*Lavandula angustifolia*), wild guinea hen weed (*Petiveria alliacea*), parsley (*Petroselinum crispum*) and thyme (*Thymus vulgaris*). The experimental plots consisted of three rows of tomato intercropped with four rows of aromatic plants. The rows were established with 1 m separation between them. Within the row, the tomato plants were established at 0.3 m distance and the aromatic plants at 0.7 m distance. The experiment was established under a design of complete randomized blocks with three replications. The blocks were separated from each other by a strip of 4 m. In the control plots only tomato plants were established without intercropping lines of aromatic species.

Seven days after transplanting (dat), imidacloprid (Confidor 350 SC, at a dose of 1 L ha⁻¹, Bayer[®]) was applied to the neck of the plant to insure protection of the crop the first 15 d after establishment. For the prevention of fungal diseases, fungicidal applications were done every 10 d alternating copper sulphate pentahydrate (Mastercop at doses of 1 L ha⁻¹, Adama[®]) and chlorothalonil (Daconil 2787 at doses of 2 kg ha⁻¹, Syngenta[®]) from day 30 to day 90 after transplant. Weed management was manual. The fertilization (kg ha⁻¹) with N:P:K was in a proportion of 140:150:150 for the period of vegetative development to fruit development, based on the recommendation for Leptosols of Yucatan (Montejo-Canul *et al.*, 2019).

Evaluation of population density of B. tabaci

The evaluation of density of *B. tabaci* was carried out at intervals of 14 d, during the period from 14 to 70 dat. The means of each sampling date were taken and averaged to integrate a single general average per treatment.

For the sampling of *B. tabaci* in the tomato leaves, nine tomato plants were selected from the centre of each experimental plot. For the morning of 7:00–8:00 am, when the white fly is least active, the adult insects were counted visually turning the leaf carefully to observe the abaxial surface of the leaf of the middle third of each plant (Góngora-Gamboa *et al.*, 2020). Afterwards, the sampled leaves were collected to count in the laboratory the number of eggs and nymphs per cm², with the help of a frame of paper of 1 cm² as sampling frame.

Evaluation of incidence and severity of viral infection in the field

The incidence and severity of the typical viral symptoms associated with begomovirus (plant stunting, yellowing of the leaves, leaf curl, atrophied plants, deformation of leaves) was recorded every 14 d, during the period of day 28 to day 70 dat. For the incidence, the number of plants with viral symptoms was counted in the total of the plants of the experimental plots. The percentage of plants with symptoms was obtained with the following formula: Incidence = [(Number of plants with symptoms) *100)] / Total of observed plants.

The degree of damage was determined by means of using six level categoric scale, modified by Caballero *et al.* (2015): level 1, asymptomatic; level 2, slight yellowing of the leaves; level 3, moderate to severe yellowing of the leaves but without the presence of foliar distortion; level 4, severe yellowing of the leaves and slight foliar distortion; level 5, severe yellowing of the leaves and severe foliar distortion; level 6, severe yellowing of the leaves, severe foliar distortion and malformation of the plant. With this data curves were constructed of the progress of the disease (ABCPE) through the method of trapezoidal integration with the formula ABCPE= $n-1\sum (xi+xi+1)/2x (ti+1-ti)$, where xi = degree of damage in each i-th evaluation; ti+1-ti = days between two evaluations, and n = the total number of observations (Agostinetto *et al.*, 2015).

To confirm the presence of begomovirus in symptomatic plants, samples were taken of leaves with characteristic symptoms in an experimental plot of each treatment. The DNA was extracted, and the genes were amplified for the detection of begomovirus using the following universal primers: Pav324 (50-GCCYATRTAYAGRAAGCCMAG-30) and Ac1154 (50-CTSAAYTTCMAAGTYTGGACG-30).

Data analysis

For the data of olfactometry an X^2 test was done with R studio comparing in each case the aroma source vs. control. The index of repellence (Kogan and Goeden, 1970) was determined using the formula IR = 2G / (G + P), where G is the number of insects in the treatment, and P is the number of insects in the control. The values of IR varied from 0 to 2: IR = 1: neutral (the aroma source is indifferent); IR > 1: attracting (the aroma source is attractive), and IR < 1: repellent. The confidence intervals (IC of 95%) were calculated for the values of IR (response of nullity = 1). The calculation of the IR indices was done for the 10 aromatic species with highest activity in the test of the olfactometer. The data were analysed with generalized linear models (GLM), using the Gaussian family function of identity linkage, with the comparison of means of Bonferroni test.

The data generated in the field crop were analysed through GLM with the family and function of specific link for each variable. For the variables of population density of *B. tabaci* in the tomato leaves, the family Gaussiana was used with function of Identity link; for the variables of area below the progress curve of the incidence and severity of begomovirus, the Gamma family was used with function link log; and for the variable of final incidence, the Poisson family was used with function link log. In all of the analyses the Bonferroni comparison of means was done (Belay *et al.*, 2012). The variable of final severity was analysed with the non-parametric test of Kruskal Wallis. All of the analyses were done using the software Infostat version 2020 (Di Rienzo *et al.*, 2018).

RESULTS AND DISCUSSION

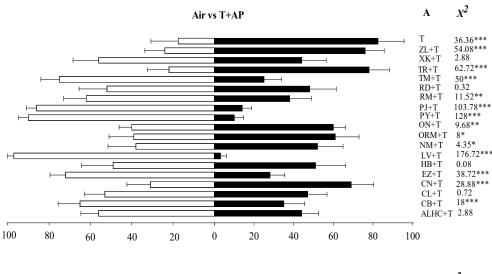
Evaluation of attraction in olfactometer

A greater attraction ($p \le 0.05$) of *Bemisia tabaci* was observed from the source of tomato aroma than from the humidified air (82.5%, X^2 =36.36). However, the attraction of *B. tabaci* decreased significantly to the source of aroma tomato + aromatic plant in comparison with the humidified air in the following cases: TM+T (25%, X^2 =50), RM+T (38%, X^2 =11.52), PJ+T (14%, X^2 =103.78), PY+T (10%, X^2 =128), LV+T (3%, X^2 =176.72) and EZ+T (28%, X^2 =38.72) (Figure 1A).

When comparing the attraction of *B. tabaci* to the tomato aroma source alone against the attraction to tomato + aromatic plant, it was observed that the attraction of *B. tabaci* to tomato decreased significantly when it was associated to the aromatic species: Lavandula angustifolia (5%, X^2 = 162), Petiveria alliacea (24%, X^2 = 54.08) and Petroselinum crispum (14%, X^2 =103.78) in Figure 1B, which suggests clear repellent effects of these aromatic species.

The repellence index (RI) was calculated for the 10 aromatic species that presented the greatest effects in the attraction of *B. tabaci* when combined with tomato (Table 2). Significant repellent activity was observed in *L. angustiolia* (IR, 0.1); however, *P. alliacea* (IR, 0.48), *P. crispum* (IR, 0.28) and *Thymus vulgaris* (IR, 0.5) also presented strong repellent tendencies (Table 2).

L. angustifolia, P. Alliaceae and P. crispum were the aroma sources which showed greatest repellent activity against B. tabaci based on the assays of olfactometry. These species have not been consigned previously as repellents against B. tabaci. In a previous study of the effect of aromatic species on B. tabaci, it was observed that C. sativum, O. basilicum and Cymbopogon citratus were highly repellent (Carvalho et al., 2017). A degree of repellent activity has also been found in Apium graveolens, Lactuca sativa and Gynura cusimbua (Zhao et al., 2014). Of the species evaluated in these studies, three were evaluated in this study, C. sativum, O. minimum and C. citratus, but none of them showed repellent effects. P. crispum and L. angustifolia were outstanding in their repellent effect. These species have VOC such as the terpenoids in their essential oils, which have shown effects against B. tabaci (Kim et al., 2011; Zhang et al., 2014). The presence of these compounds may have caused, in part, the repellent effect in the laboratory bioassays in this study.



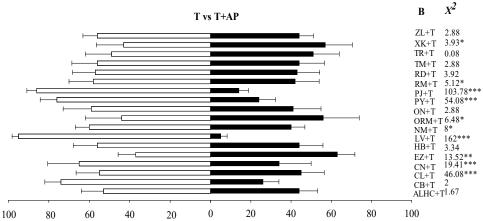


Figure 1. Percentage of the response of *Bemisia tabaci* to the odor source: (Panel A) tomato + aromatic plant (T + AP) relative to the humidified air, (Panel B) sole tomato (T) relative to tomato + aromatic species (T + AP). The assessment was carried out by olfactometry under laboratory conditions. Tests of X²: *<0.05; **<0.01 ***<0.001. ZL, lemon grass; XK, wild basil; TR, lemon balm; TM, thyme; RD, rue; RM, rosemary; PJ, parsley; PY, guinea hen weed; ON, oregano; ORM, Mexican oregano; NM, neem; LV, lavender; HB, spearmint; EZ, epazote; CN, citronella; CL, cilantro; CB, chives; ALHC, common basil.

Population density of B. tabaci in the field

The tomato crop showed no significant difference in the field regarding population density of adults ($X^2_{(4, 650)} = 0.39$, p = 0.862) and of eggs ($X^2_{(4, 648)} = 1.45$, p = 0.21) of *B. tabaci* among the treatments (tomato intercropped with aromatic species) and the control (tomato alone). However, a tendency was observed of a lower population density of *B. tabaci* in the tomato intercropped with the aromatic species *L. angustifolia* and *P. crispum* (Figure 2A; 2B).

Table 2. Index of repellence (IR) measured through the response of adults of *Bemisia tabaci* to the odor source of crushed tomato leaves and tomato + aromatic plant in bioassays of olfactometry under laboratory conditions.

Aromatic species	T vs T+PA RI <u>+</u> EE	
T. vulgaris (TM) L. angustifolia (LV) P. citrosum (CN) R. officinalis (RM) O. micranthum (XK) D. ambrosioides (EZ) C. sativum (CL) P. alliacea (PY) A. indica (NM)	0.88±0.25 ab 0.10±0.06 a 0.68±0.31 ab 0.84±0.24 ab 1.14±0.26 ab 1.26±0.17 b 0.9 ±0.23 ab 0.48±0.16 ab 0.8 +0.13 ab	
P. crispum (PJ)	0.28±0.09 ab	

Means (\pm SE) with different letters indicate statistical differences (GLM, Bonferroni $p \le 0.05$).

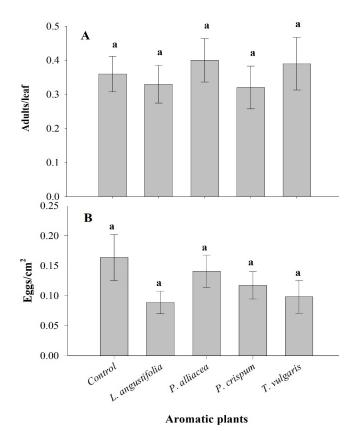


Figure 2. Average number of adults of *Bemisia tabaci* per tomato leaf (A) and number of eggs of *B. tabaci* per cm² of leaves (B) of tomato intercropped with *Lavandula angustifolia, Petiveria alliacea, Petroselinum crispum* or *Thymus vulgaris*. The bars indicate averages \pm standard error. Different letters over the bars indicate statistical difference (GLM, Bonferroni, $p \le 0.05$).

Although the presence of aromatic plants intercropped with the tomato did not have significant effects, a clear tendency was observed of the effect of *L. angustifolia* and *P. crispum* in reducing the density of eggs and adults. Phytochemical studies show that the organic volatiles of *L. angustifolia* present a high amount of terpenoids, which have repellent effects against various species of phytophagous insects (Zhang *et al.*, 2014; Han *et al.*, 2020).

In this study, the density and distribution of the aromatic plants in the tomato crop was due in part to aspects of crop management and the intensity of labour for maintenance of the agroecosystem; since the established aromatic plants also require care, such as weeding and irrigation. The lack of effect of the aromatic plants was possibly due to a low planting density compared to the tomato. It has been reported that the density and distribution of the aromatic plants influence their effect on the communities of phytophagous insects (Ben Issa *et al.*, 2016). Crops with aromatic plants intercropped in a proportion higher than 50% generate greater effects of repellence to the pests (Li *et al.*, 2021). Furthermore, the aromatic species included in the field experiment are shorter in height and with lower growth velocity than the tomato plants, which also could have affected the efficiency of the aromatic plants as emitters of volatiles and consequently in the repellence of adults of *B. tabaci*. It has also been reported that the production of VOC in the aromatic plants is related to the amount and intensity of light (Ascrizzi *et al.*, 2018; Thakur and Kumar, 2020).

Viral infection of tomato

The presence of begomovirus was confirmed through the obtainment of DNA samples from leaves corresponding to plants with viral symptoms in all of the treatments. The electrophoresis in gel of the products obtained from the PCR confirmed the presence of begomovirus in the plants sampled.

In general, the incidence and severity of begomovirus was low in all of the treatments (Table 3). Although there was no significant difference among treatments ($X^2_{(4, 10)} = 0.17$, p = 0.95) in the area below the progress curve of incidence, the final incidence tended to be lower in the tomato intercropped with *L. angustifolia* (21±2 %) compared with the tomato without intercropping of aromatic plants (29.5±2.7%).

Table 3. Means (±standard error) of the area under the progress curve (AUPC) of the incidence and severity, final incidence and final severity of viral symptoms associated with the presence of *Bemisia tabaci* in the tomato intercropped with different aromatic species.

Aromatic	Incidence	Final	Severity	Final
Species	AUPC	incidence	AUPC	severity
Control L. angustifolia P. alliacea P. crispum T. vulgaris	714.8 ±194.4a	29.5±2.7ab	116.6±9.3a	3.5±0.1a
	715.7±224.8a	21.0±2.7b	103.7±9.5a	3.4±01a
	910.0±285.8a	37.7±3.5a	116.9±10.8a	3.6±0.2a
	855.7± 268.8a	28.0±3.1ab	103.0±9.5a	2.3±1.2a
	665 ± 255.8a	28.8±3.7ab	72.7±8.2a	2.9±0.9a

Different letters within the same column indicate statistical difference (GLM, Bonferroni $p \le 0.05$).

In regard to the severity of the symptoms in all of the treatments, the plants manifested degrees of damage between 2.3 to 3.6. There was no significant statistical difference among treatments in the area below the progress curve of severity ($X_{(4,10)}^2 = 3.45$, p = 0.05) or in the final severity (df = 4, H = 2.13, p = 0.7).

The incidence (less than 40 %) and severity of viral symptoms (grade 3 to 4 of damage) were low in the tomato crop. Nevertheless, a tendency to reduce the damage from virosis was observed in the effect of *L. angustifolia*, which suggests that despite the absence of significant results, the repellent effect of *L. angustifolia* was observed in the reduction of mobility of *B. tabaci* and consequently in the decrease of symptoms associated with the virus (Erland *et al.*, 2016; Ghosh and Ghanim, 2021). The presence of VOC in the environment can alter not only the capacity of *B. tabaci* to colonize their host, but also can affect the capacity to transmit the virus. In this regard, it has been documented that the alteration in the feeding behaviour of the vectors of phytopathogenic virus mediates the acquisition and inoculation of virus (Zhou *et al.*, 2018).

One important aspect to consider in crops where intercropped aromatic plants are established to repel phytophagous insects is to know well the conditions and growth dynamic of the aromatic species to be included in the agroecosystem, due to the fact that they present problems of adaptation, as slow growth, or damage from phytoparasites, in the area of the crop. This would affect the amount of VOC emitted and consequently their efficiency as repellent of phytophagous insects (Chi *et al.*, 2021).

CONCLUSIONS

In the laboratory tests with the use of bioassays in olfactometer, the volatiles emitted by crushed leaves of lavender (*Lavandula angustifolia*), guinea hen weed (*Petiveria alliacea*), parsley (*Petroselinum crispum*) and thyme (*Thymus vulgaris*) caused significant reduction in the attraction of *Bemisia tabaci* to tomato.

Although these aromatic species did not have significant effects, a tendency of the effect of lavender (*L. angustifolia*) was observed in the suppression of population density of adults and eggs of *B. tabaci* in the tomato crop on the field. As well as the final incidence of viral symptoms associated with the presence of begomovirus according to the molecular analysis carried out on the symptomatic plants.

ACKNOWLEDGEMENTS

To the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the Master scholarship awarded to Angel Manuel Herrera Gorocica.

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