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Bioactivity and Repellency of Some Plant Extracts Against the Tomato Leaf Miner *Tuta absoluta* (Meyrick 1917) (Lepidoptera: Gelechiidae).

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ABSTRACT

This study aimed to evaluate the bioactivity of 8 plant oil extracts namely garlic, clove, basil, peppermint, eucalyptus, jojoba, wormwood and rose separately and in combination with *Bacillus thuringiensis* (*B.t.*) against the 1st larval instar of the tomato leaf miner *Tuta absoluta* under laboratory conditions. The highest larval mortality was recorded after application of garlic, clove, eucalyptus and then wormwood oils. *B.t.* potentiated the activity of the tested plant extracts against the larvae of *T. absoluta* with few exceptions. This is judged from the significant reduction in the lethal concentrations where the highest larval mortality after mixing with *B.t*. occurred after application of clove, eucalyptus, wormwood and garlic oils. The tested plant extracts significantly affect the growth rate of the larvae that survived but in varying degrees. Clove oil was the most effective in reducing the larval growth rate (11.04%) while rose oil was the least effective (2.26%). The larval duration of the surviving individuals was significantly retarded being 14.73±1.41 and 14.61±1.27days after treatment with clove and garlic oils, respectively. The pupal duration as well followed the same trend. Garlic oil and clove oil caused significant reduction in pupal weight and the reduction rate was -30.03 and -20.7%, respectively. This is related to the repellent effect of the extracts which reduced feeding. It appears that garlic, clove, eucalyptus and peppermint oil extracts are highly repellent, while jojoba, rose and wormwood had less repellent effects. These results indicate that plant extracts showed promise for the control of *T. absoluta* larvae on tomato plants, since it exhibits toxicity toward this insect at 1000 - 2000 ppm concentrations separately or mixed with *B.t*. they also affect the insect's development by retarding the larval and pupal durations and pupal weight. The strong repellent effects of some of these extracts minimize the rate of plant damage.

Keywords: *Bacillus thuringiensis* - Plant extracts – Bioactivity- Repellency - *Tuta absoluta*

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INTRODUCTION

Tomato (*Solanum lycopersicum*) is one the most important economic vegetables in Egypt and it is a host plant to about 200 species of arthropods (Anonymous 2001). During the second half of 2009, the tomato cultivations in Egypt faced the invasion of a newly dangerous insect pest namely *Tuta absoluta* (Tamerak 2011; Gaffar 2012) and which may reduce the productivity up to 100%. The damage is caused by the larvae, which feed and grow on soft tissues such as leaves, shoots and fruits from the aerial part of the plant at any stage of tomato growth.

Alternative pest management using the ecofriendly means and easily biodegradable plant extracts with natural insecticidal activity has increased in recent years to control insect pests without adverse effects on the environment (Belmain et al. 2001). These plant extracts are considered to be an alternative to conventional pesticides because of their low toxicity to warm-blooded mammals as well as their high volatility.

One of the most known plant extracts in recent decades is neem (Morgan 2004), and several commercial formulations of it are now available worldwide. Also, different plant extracts and essential oils were used in pest management in different crops against various pests and caused significant reduction in insect population e.g. various natural products from the genus *Artemisia* (Saleh 1984; Dimetri et al. 1996; Wang et al. 2006; Gonzalez-Coloma et al. 2011; Martín et al. 2011), mustard and clove oils (Sabbour & Abd-El-Aziz 2010), clove and bitter orange (Ebadah, Shalaby & Moawad 2016) and lemon grass extract, garlic, eucalyptus, rue, anise and basil oils (Hussein et al. 2014).

The present work aimed to evaluate the bioactivity of leaf ethanolic extracts of eight species of plants namely garlic (*Allium sativum*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus camaldulensis*), peppermint (*Mentha spicata*), basil (*Ocimum basilicum*), wormwood (*Artemisia annua*), jojoba (*Simmondsia chinensis*) and rose (*Rosa damascena*). Investigations were conducted in two phases. In the first phase, the effect of these extracts was evaluated alone, while in the second phase studies on the effects of a combination of *B.t.* with the plant extracts against the first larval instar of the tomato leaf miner *T. absoluta* were carried out under laboratory conditions.

MATERIAL & METHODS

Insect colony:

A colony of *T. absoluta* was established using larvae collected from tomato cultivations, at Al- Almeen road- Mersa Matrouh governorate). The colony was maintained in Pests and Plant Protection Laboratory, National Research Centre under appropriate conditions (27±2°C, 65±10 % RH and 14-hour photophase). Leaves of tomato plants were used to feed the insect during the larval stage and a solution of 10% honey during the adult stage. The insects were maintained in wood cages (50x50x60 cm) with narrow meshes only at the front and on the top. The cages are provided with pots of tomato plants for oviposition. The deposited eggs on tomato leaves were separated daily to get the first larval instar. In this study the first larval instars were used to evaluate the toxicity of different plant extracts under laboratory conditions.

Toxicity of some plant extracts:

Ethanolic oil extracts of garlic, clove, eucalyptus, peppermint, basil, wormwood, jojoba and rose were used. These extracts were obtained from Dr. Mona M. El-Dien, natural insecticides laboratory, pests and plant protection department, NRC. These extracts tested to evaluate their larvicidal effects at four concentrations 2000, 1000, 500 and 250 ppm using leaf-dip bioassay method (Cahill et al. 1996, Nilahyane et al. 2012). The tomato leaves were washed, dried then dipped in 100 ml of prepared concentrations of the extract after mixing with 2 ml of ethanol 98% or chloroform with addition of Tween-20 as emulsifier then stirred gently for 5 minutes and then left to dry for 5 minutes at an ambient temperature on a nonabsorbent surface. After evaporation of the access water, three leaves were then placed in a Petri dish containing moist filter paper; to maintain moisture, the petiole of each leaflet was wrapped in moistened cotton wool. Ten larvae were starved for 12 hours then introduced into treated tomato leaves, with the control treated with water. Five replicates were used for each treatment. The larval mortality was assessed daily for six days with the aid of a binocular stereo microscope (Ferrari de Brito et al. 2015) to evaluate the toxicity of the tested plant extracts against the larvae.

Toxicity of combination of B.t. and plant extracts:

In a second series of experiments, the toxicity of the commercial formulation of *B.t.* (Dipel-2X) was tested using LC_{50} in a combination with the serial concentrations of the tested plant extracts. The lethal concentration (LC_{50}) of Dipel-2x (32000 IU/mg) against the 1st larval instar of *T. absoluta* has been determined as 86.02×10^3 IU/ml in preliminary experiments by Shehata (2015).

For this purpose, 0.27 gm of Dipel-2X powder was dissolved in 100 ml distilled water to give the value of LC_{50} of *B.t.* (Dipel-2x), then mixed with each of the concentrations of each plant extract. The mixtures were prepared in 100 ml water for bioassay on the larvae using leaf-dip bioassay method as mentioned above. The larval mortality was assessed daily for six days to evaluate the synergistic effects of the tested plant extracts after mixing with *B.t.* against the larvae of *T. absoluta*.

Effects of plant extracts on different biological aspects of T. absoluta:

To evaluate the effects of the plant extracts on the biology of *T. absoluta*, five replicates of the first larval instar (10 larvae starved for 12 hours/replicate) were reared or fed on tomato leaves treated with the LC_{50} of the tested plant extracts as a contaminated food for 4 days then replaced with untreated leaves for 10 days to complete the larval stage development under laboratory conditions (27±2°C, 65±10 % RH). The leaf-dip bioassay method was adopted. The following biological aspects were:

- **Growth rate of larvae** according to Waldbaur (1968),

$$\text{Growth rate (GR)} = G \;/\; T \times A$$

G = Weight gained of larvae during feeding period /(mg).
T = Duration of feeding period (10) days.
A = Mean weight of larvae during the feeding period

$$A = \text{Initial weight} - \text{Final weight} / 2$$

- **Larval, pupal duration and pupal weight (48 hours old) resulting from treated 1st larval instar.**

Newly hatched 1st instar larvae were allowed to feed on treated tomato leaves. The larval and pupal duration as well as the pupal weight were determined.

- **Repellency of plant extracts**, according to Zapata & Smagghe (2010).

A choice bioassay system was used to evaluate repellency of the tested plant extracts. Clean tomato leaves disks (5 cm) were dipped in 100 ml of distilled water mixed with LC_{50} of each tested oil and solvent, stirred gently for 5 minutes and then left to dry for 5 minutes at ambient temperature on a nonabsorbent surface. After evaporation of the access water, two disks of leaves (the first was treated while, the second was untreated) were then placed separately in a Petri dish (25 cm) containing moist filter paper. Ten larvae of the 1st larval instar were put into each Petri dish and the lid was sealed with parafilm. Five replicates (10 larvae/replicate) were run for each tested oil under the same environmental conditions described for the rearing. The numbers of insects on the two disks were recorded after 2 and 24 hours from the beginning of the test.

Percentage of repellency (PR) was calculated according to Nerio, Olivero-Verbel & Stashenko (1990) as follows:

$$\text{Percentage of repellency (PR)} = (C - T / C + T) \times 100$$

C = No. of larvae on the untreated disk.
T = No. of larvae on the treated disk.

Statistical analysis

Data obtained were submitted to analysis of variance, one way ANOVA was used (F- test) to compare the results and then differences were considered significant at $p < 0.05$ level using SPSS program. The bioassay data were subjected to probit analysis program (Microsoft office, 2003) to determine the mean of accumulative mortality percentage / day and these data were used to determine LC_{50}, LC_{95} at 95% confidence limits.

RESULTS

***Toxicity of some plant extracts*:**

The results given in table (1) show the accumulative daily mortality percentage of the first larval instar of *T. absoluta* after treatment with different concentrations (2000, 1000, 500 and 250 ppm) of eight plant extracts under laboratory conditions. The data showed varying larval susceptibility depending on the tested plant extract, applied concentration and exposure time.

In the case of garlic oil, data in table (1) revealed that this extract gave the highest larval mortality at all tested concentrations. The larval mortality percentages were 26.7, 23.33 and 6.7% after application of 2000, 1000 and 500 ppm 2 days post treatment, respectively, with no mortality at 250 ppm. Increase of the exposure time to 4 days increased the mortality percentage to be 63.33, 50, 30 and 23.33% with 3.33 % mortality in untreated larvae while the highest larval mortality percentages were 76.7, 63.33, 43.33 and 26.7% 6 days post-treatment with the tested concentrations, respectively, compared with the mortality in control group that was 10 %. So there was a highly significant difference in the susceptibility of the 1st larval instar of *T. absoluta* to garlic extracts at 6 days as compared with the control ($P<0.05$; $F= 53.8$).

In the case of clove oil, the results in table (1) showed that the effect was very low 2 days post-treatment, where the mortality record at both 2000 and 1000 ppm was 26.7 and 6.7%, respectively, with no mortalities at the other concentrations. Four days after treatment, the mortality percentages increased to 60, 33.33, 33.33 and 10 % at the tested concentrations, respectively. At 6 days post treatment, the highest record of mortality was 66.7% after treatment with 2000 ppm of clove oil then decreased to 56.7 and 43.33 after treatment with 1000 and 500 ppm, respectively, with no significant effects between them. While, the lowest mortality percentage was 26.7% after application of 250 ppm compared with 10 % in the control group. Statistical analysis showed significant differences in treatments with clove oil as compared with the control group ($P<0.05$; $F= 33.7$).

With peppermint oil, the 1st larval instar of *T. absoluta* showed more susceptibility of the larvae to the high concentrations as recorded in table (1). After 2 days of application, the tested concentrations 2000 and 1000 ppm caused mortalities 26.7 and 16.7%, respectively, with no mortalities at the lower concentrations. The mortality percentage increased after 4 days post-treatment to 40, 26.7, 16.7 and 3.33% while, the highest mortality percentage was 56.7 and 36.7% 6 days after treatment with 2000 and 1000 ppm, respectively with highly significant effects as compared with the control group ($P<0.05$; $F= 18.9$). While the mortality percentages decreased to 20 and 13.33% after application of 500 and 250 ppm, respectively with no significant differences as compared with the control group.

In the case of basil oil, the results in table (1) showed highly significant effects in larval mortalities after application of 2000 and 1000 ppm as compared with the control group. The mortality percentages were 20, 40 and 50 % at 2000 ppm and then decreased to 13.33, 33.33 and 43.33 % at 1000 ppm after 2, 4 and 6 days from treatment. While, application of 500 and 250 ppm of basil oil have no significant effects compared with the control group, where the mortality percentages were 6.7, 10 and 16.7% at 500 ppm then decreased to 3.33, 3.33 and 13.33 at 250 ppm as compared with 0, 3.3 and 10 % mortalities in the control group after 2,4 and 6 days from treatment, respectively.

In the case of wormwood oil, the larval mortality caused by this extract after 2 days of treatment was very low at all tested concentrations then increased with increase of the exposure time. The highest percentages of mortality were 16.7, 30 and 60 % after treatment with 2000 ppm, 2, 4 and 6 days after treatment, respectively, while in larvae treated with 1000 ppm the mortality decreased to 3.33, 20 and 43.33% after 2, 4 and 6 days of treatment, respectively with highly significant effects in these treatments as compared with the control ($P<0.05$, $F= 20.2$). Application of 500 ppm caused no mortality 2 days post-treatment then increased to 13.33 and 30 % with increasing of the exposure time to 4 and 6 days, respectively. Also, at 250 ppm no mortalities were observed 2 days post-treatment then increased to 3.33 and 16.7 % with increasing of the exposure time to 4 and 6 days, respectively and so this concentration has insignificant effects on the larval mortality as compared with the control group where the mortality percentage in control was 10 % 6 days post treatment.

Data on the activity of the eucalyptus oil in table (1) indicate that, the mortality percentage was 20, 16.7 and 6.7 % at 2000, 1000 and 500 ppm 2 days post-treatment, respectively, with no mortality at 250 ppm. The mortality increased to 43.33, 40, 23.33 and 13.33% with increase of the exposure time in the tested concentrations to 4 days, respectively. The highest mortality percentage was recorded 6 days post-treatment being 63.33, 50, 36.7 and 23.33% at the tested concentrations, respectively. Significant effects were observed for the mortalities at all tested concentrations as compared with the control ($P<0.05$; $F= 31.5$).

In the case of the rose oil (table 1) the larval susceptibility is very weak compared with the other tested plant extracts. The mortality percentage was 16.7, 26.7 and 43.33% at 2000 ppm then decreased to 6.7, 20 and 33.33% after treatment with 1000 ppm 2, 4 and 6 days post-treatment, respectively with low significant differences between the larval susceptibility to these concentrations compared with the control. While, insignificant effects were recorded between mortalities of the larvae treated with 500 and 250 ppm of rose oil compared with the control group, where the mortality percentages were 10, 13.33 and 20 % at 500 ppm and 0, 3.33 and 10 % at 250 ppm and the untreated control larvae 2,4 and 6 days post-treatment, respectively.

Table (1) also showed the larval susceptibility to different concentrations of Jojoba oil. All tested concentrations caused low mortality percentages 2 days post-treatment where the mortality ranged from 20 to 3.3 % after treatment with the tested concentrations ranging from 2000 to 250 ppm, respectively. While the mortality percentages increased with increasing of the exposure time to 4 days and the mortality percentage was 26.7, 23.33, 10 and 10 % and the highest mortality percentages were recorded at 6 days after treatment to be 53.33, 40. 36.7 and 20 % at the four tested concentrations, respectively. Significant differences were observed between larval mortalities at 2000,1000 and 500 ppm of jojoba oil as compared with the control group ($P<0.05$; $F= 24.9$) but no significant effects were observed between the mortalities at 250 ppm and the untreated control.

From the data given in table (1), the lethal values of LC_{50} and LC_{95} were computed through probit analysis program at 95% confidence limits and the values are given and illustrated in Figs. (1&2). According to the LC_{50} values/ppm, the larvae of *T. absoluta* were more susceptible to garlic (680.60 ppm) than clove (774.5 ppm), eucalyptus (1008.6 ppm), wormwood (1297.8 ppm), jojoba (1612.2 ppm), peppermint (1636.2 ppm), basil (1987.1 ppm) and finally rose (2527.8 ppm).

According to the LC_{95} values/ppm, the larvae of *T. absoluta* were more susceptible to garlic (9262.2 ppm) than clove (20397.7 ppm), peppermint (21603.9 ppm), wormwood (22324.5 ppm), eucalyptus (25272.5 ppm), basil (27820.6 ppm), rose (55973.4 ppm) and finally jojoba (92418.1 ppm).

Toxicity of combinations of B.t. and plant extracts:

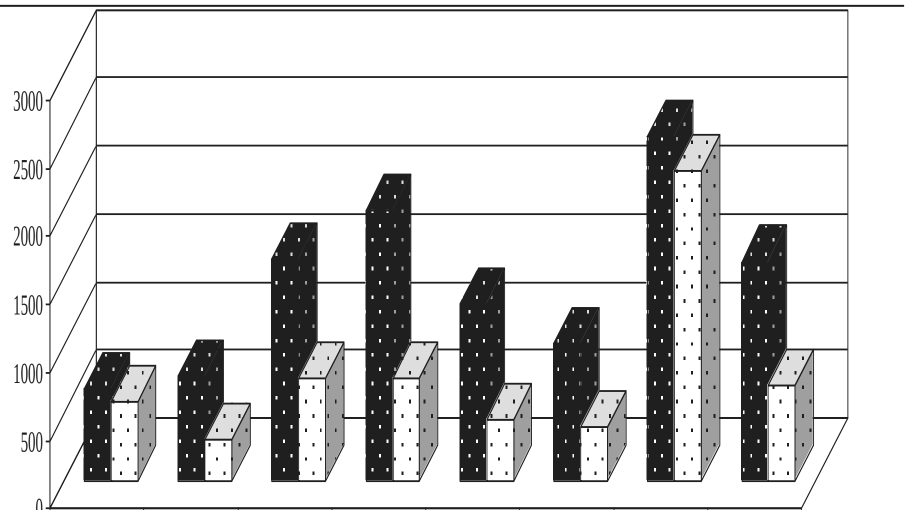
Potency of combinations of botanical extracts and biological insecticides was investigated in an attempt to determine their compatibility. It is known that the biological insecticide *B.t.* is an important biotic entity that aids in regulation of insect population and keeping it before the damaging level and certainly, they have much to offer for insect control separately or in combinations. For this purpose, the lethal concentration LC_{50} of commercial product Dipel-2X (*B.t.*) has been evaluated in combinations with different concentration of plant extracts for its potency against the 1st larval instar of *T. absoluta*.

Data presented in table (1) showed that in a combination of garlic oil and *B.t.* the effects of *B.t*. on larval mortality was very low compared with garlic oil when used singly. The highest mortality percentage was 76.7 % at 2000 ppm of garlic oil combined with *B.t*., then decreased to 70% at 1000 ppm of garlic combined with *B.t*. 6 days post-treatment. The statistical analysis showed highly significant differences in mortalities at different concentrations compared with the control group (33.33%) at application of *B.t*. when used singly. The mortality percentages in larvae treated with 500 and 250 ppm of garlic oil combined with *B.t.* were 46.7 and 36.7%, respectively, 6 days post-treatment, with insignificant effects recorded compared with the control group. The values of LC_{50} and LC_{95} at 95% confidence limits were 586 and 9505 ppm, respectively, Figs. (1 & 2).

Increase of the larval mortalities was recorded in varying degrees after treatment with combinations of the clove, peppermint, basil, wormwood, eucalyptus and jojoba with *B.t*. Increase of the exposure time to 6 days caused high significant efficiency in reducing larval population and the mortality percentage reached to 80 % as with clove oil (2000 ppm + *B.t*.).

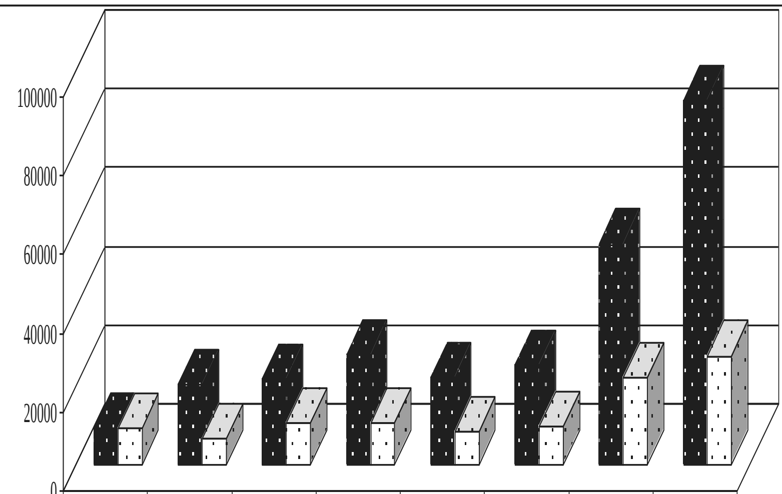
Analysis of the data according to the calculated values of LC_{50}/ppm, showed that the larvae of *T. absoluta* were more susceptible to clove (308.6 ppm) then eucalyptus (396.1 ppm), wormwood (450 ppm), garlic (586 ppm), jojoba (708.02 ppm), basil (753.6 ppm), peppermint (754.9 ppm) and finally rose oils (2277 ppm) (Fig. 1).

Meanwhile, according to the LC_{95} values/ppm, the results show clearly the same trend with few exceptions. The larvae of *T. absoluta* were more susceptible to clove (6778.3 ppm) than wormwood (8451.9 ppm), garlic (9505 ppm), eucalyptus (9938 ppm), peppermint (10725.5 ppm), basil (10727.8 ppm), rose (22380.5 ppm) and finally jojoba oil (27737.8 ppm) (Fig. 2).These results indicate that the application of some plant extracts with *B.t*. may be a safe and effective means for controlling *T. absoluta*.



	Garlic	Clove	Peppermint	Basil	Wormwood	Eucalyptus	Rose	Jojoba
■ LC50 of plant extracts alone	680.6	774.5	1636.2	1987.1	1297.8	1008.6	2527.8	1612.2
□ LC50 of plant extracts combined with B.t.	586	308.6	754.9	753.6	450	396.1	2277	708.02

Fig. 1. Potency of combination of plant extracts and *B. thuringiensis* compared with plant extracts alone against the 1st larval instar of *Tuta absoluta* depending on LC_{50} values/ppm



	Garlic	Clove	Peppermint	Basil	Wormwood	Eucalyptus	Rose	Jojoba
■ LC95 of plant extracts alone	9262.2	20397.7	21603.9	27820.6	22324.5	25272.5	55973.4	92418.1
□ LC95 of plant extracts combined with B.t.	9505	6778.3	10725.5	10727.8	8451.9	9938	22380.5	27737.4

Fig. 2. Potency of combination of plant extracts and *B. thuringiensis* compared with plant extracts alone against the 1st larval instar of *Tuta absoluta* depending on LC_{95} values/ppm

Table 1. Potency of various plant extracts singly and in combination with *B. thuringiensis* against the first larval instar of *Tuta absoluta*

Concentration of plant extract (ppm)	Days post-treatment	% of larval accumulative mortality															
		Garlic		Clove		Peppermint		Basil		Wormwood		Eucalyptus		Rose		Jojoba	
		alone	with *B.t.*	alone	with *B.t.*	alone	with *B.t.*	alone	with *B.t.*	alone	with *B.t.*	alone	with *B.t.*	alone	with *B.t.*	alone	with *B.t.*
2000	2	26.7	13.33	26.7	23.33	26.7	26.7	20	13.33	16.7	30	20	16.7	16.7	16.7	20	30
	4	63.33	76.7	60	56.7	40	53.33	40	43.33	30	53.33	43.33	70	26.7	33.33	26.7	40
	6	76.7 ^{a}	76.7 ^{a}	66.7 ^{a}	80 ^{a}	56.7 ^{a}	63.33 ^{a}	50 ^{a}	66.7 ^{a}	60 ^{a}	73.33 ^{a}	63.33 ^{a}	80 ^{a}	43.33 ^{a}	53.33 ^{a}	53.33 ^{a}	66.7 ^{a}
1000	2	23.33	16.7	6.7	10	16.7	0	13.33	10	3.33	23.33	16.7	13.33	6.7	10	10	23.33
	4	50	60	33.33	40	26.7	36.7	33.33	40	20	40	40	46.7	20	26.7	23.33	40
	6	63.33 ^{b}	70 ^{a}	56.7 ^{ab}	80 ^{a}	36.7 ^{b}	50 ^{a}	43.33 ^{a}	56.7 ^{ab}	43.33 ^{b}	63.33 ^{ab}	50 ^{ab}	63.33 ^{b}	33.33 ^{a}	36.7 ^{b}	40 ^{b}	56.7 ^{ab}
500	2	6.7	13.3	0	10	0	0	6.7	3.33	0	13.33	6.7	13.33	10	6.7	3.33	6.7
	4	30	30	33.33	46.7	16.7	26.7	10	33.33	13.33	40	23.33	40	13.33	23.33	10	33.33
	6	43.33 ^{c}	46.7 ^{b}	43.33 ^{b}	60 ^{b}	20 ^{c}	50 ^{a}	16.7 ^{b}	46.7 ^{bc}	30 ^{b}	56.7 ^{b}	36.7 ^{bc}	63.33 ^{b}	20 ^{c}	33.33 ^{b}	36.7 ^{b}	46.7 ^{b}
250	2	0	0	0	6.7	0	0	3.33	3.33	0	3.33	0	0	0	0	3.3	10
	4	23.33	16.7	10	30	3.33	20	3.33	30	3.33	30	13.33	13.33	3.33	16.7	10	20
	6	26.7 ^{d}	36.7 ^{b}	26.7 ^{c}	43.33 ^{c}	13.33 ^{c}	33.33 ^{b}	13.33 ^{b}	36.7 ^{c}	16.7 ^{d}	36.7 ^{c}	23.33 ^{cd}	36.7 ^{c}	10 ^{c}	33.33 ^{b}	20 ^{c}	30 ^{c}
Control (H_2O)	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	4	3.33	13.33	3.33	13.33	3.33	13.33	3.33	13.33	3.33	13.33	3.33	13.33	3.33	13.33	3.33	13.33
	6	10 ^{e}	33.33 ^{b}	10 ^{d}	33.33 ^{c}	10 ^{c}	33.33 ^{b}	10 ^{b}	33.33 ^{c}	10 ^{d}	33.33 ^{c}	10 ^{e}	33.33 ^{c}	10 ^{c}	33.33 ^{b}	10 ^{c}	33.33 ^{c}

Means followed by similar letters within the same vertical column are not significantly different at 0.05 level.

Effects of plant extracts on different biological aspects of T. absoluta:

- Effects of plant extracts on growth rate of 1st larval instar.

The effect of plant extracts on the growth rate of the larvae after feeding on treated leaves is given in table (2). The weight of the larvae before treatment ranged from 0.11±0.02 to 0.14±0.01 mg. The final weight of the larvae after 10 days was 0.78±0.12, 0.88±0.04, 0.87±0.21, 0.91±0.1, 0.95±0.04, 1.06±0.14, 0.93±0.23 and 0.92±0.11mg in the larvae fed treated tomato leaves with garlic, clove, eucalyptus, peppermint, wormwood, rose, basil and jojoba oils, respectively compared to1.27±0.14 mg in the control group.

On the other hand, the gained weight in untreated larvae was 1.14 ± 0.1 mg, while the gained weight in treated larvae was significantly reduced in all treatments compared with the control. The gained weight was 0.66±0.11, 0.74±0.05, 0.75±0.2, 0.8±0.05, 0.84±0.05, 0.94±0.1, 0.82±0.13 and 0.81±0.17 mg in larvae treated with garlic, clove, eucalyptus, peppermint, wormwood, rose, basil and jojoba oil extracts, respectively. This indicates that the percentages of reduction in the larval weight after 10 days were 42.11, 35.09, 34.21, 29.82, 26.32, 17.54, 28.07 and 28.95 %., respectively. Statistically no significant differences were observed between the gained weight in the larvae fed on treated tomato leaves with garlic, clove and eucalyptus oil extracts. In addition, highly significant differences were observed between the gained weight of larvae fed on treated tomato leaves with all plant extracts compared with the control group.

Also, data in table (2) demonstrated the effect of the plant extracts in reducing the larval growth rate. Application of clove oil led to highest reduction in the larval growth rate (11.04%), while the use of rose oil led to lowest reduction in the rate of larval growth (2.26%). Garlic, clove and eucalyptus oil extracts reduced significantly the growth rate where the percentages were 9.82, 11.04 and 6.75 % compared with the control. In addition, no significant effects were observed between effects of peppermint, wormwood, rose, basil and jojoba oil extracts on the growth rate of treated larvae as compared with the control, where the percentages of growth rate reduction were 4.91, 3.07, 2.26 , 3.07 and 3.68%, respectively.

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Table 2. Potency of various plant extracts on the larval growth of the first larval instar of *Tuta absoluta* under laboratory conditions

Plant extracts	Weight of the larvae / mg (n= 50) (Five replicates, 10 larvae for each)					
	Initial weight before treatment (at zero time)	Final weight (10 days post-treatment)	Gained weight (G) (weight of larvae during feeding period)		Growth rate (GR)	
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*% of larval weight reduction*	*Mean ± SD*	*% of GR reduction*
Control	0.13 ± 0.02 [a]	1.27 ± 0.14 [a]	1.14 ± 0.1 [a]	-	0.163 ± 0.02 [a]	-
Garlic	0.12 ± 0.04 [a]	0.78 ± 0.12 [b]	0.66 ± 0.11 [b]	42.11	0.147 ± 0.04 [bc]	9.82
Clove	0.14 ± 0.01 [a]	0.88 ± 0.04 [bc]	0.74 ± 0.05 [bc]	35.09	0.145 ± 0.01 [b]	11.04
Eucalyptus	0.12 ± 0.05 [a]	0.87 ± 0.21 [bc]	0.75 ± 0.2 [bc]	34.21	0.152 ± 0.04 [bc]	6.75
Peppermint	0.12 ± 0.02 [a]	0.91 ± 0.1 [c]	0.8 ± 0.05 [c]	29.82	0.155 ± 0.01 [acd]	4.91
Wormwood	0.11 ± 0.05 [a]	0.95 ± 0.04 [c]	0.84 ± 0.05 [cd]	26.32	0.158 ± 0.01 [ad]	3.07
Rose	0.12 ± 0.01 [a]	1.06 ± 0.14 [d]	0.94 ± 0.1 [d]	17.54	0.159 ± 0.03 [ad]	2.26
Basil	0.11 ± 0.02 [a]	0.93 ± 0.23 [c]	0.82 ± 0.13 [c]	28.07	0.158 ± 0.01 [ad]	3.07
Jojoba	0.11 ± 0.03 [a]	0.92 ± 0.11 [c]	0.81 ± 0.17 [c]	28.95	0.157 ± 0.01 [ad]	3.68
	L.S.D = 0.03	L.S.D = 0.09	L.S.D = 0.1		L.S.D = 0.01	
	F = 0. 41	F = 4.32	F = 2.9		F = 1.23	

Means followed by similar letters within the same vertical column are not significantly different at 0.05 level.

- **Effects of plant extracts on larval duration, pupal weight and pupal duration) surviving after treatment of 1st larval instar.**

In this study the larval duration, weight of pupae and pupal duration of pupae resulting from larvae fed previously on tomato leaves treated with the tested plant extracts was evaluated to explore its role as anti-feedants or feeding deterrents.

Data given it table (3) showed that the larval duration in the control larvae was 11.15±0.39 days; this was prolonged for those fed on leaves treated with the tested oil extracts of garlic, clove, eucalyptus, peppermint, wormwood, basil, jojoba and rose oil being 14.61±1.27, 14.73±1.41, 13.13±1.26, 12.52±1.44, 12.48±1.39, 12.25±1.27, 12.23±0.31 and 11.58±0.98 days, respectively. Statistical analysis showed high significant increase ($P<0.05$) in larval duration for those previously fed on leaves treated with garlic oil (+31.03%), clove oil (+32.1%), eucalyptus oil (+17.76%) and peppermint oil (+12.29%) as compared with the control. No significant changes in larval development for those previously fed on leaves treated with wormwood, rose, basil and jojoba oil extracts.

On the other hand, garlic and clove oil extracts caused a significant reduction in the pupal weight being 2.4±0.26 and 2.72±0.19 mg, respectively compared with the control, but with insignificant reduction in the pupal weight previously treated as larvae with eucalyptus, peppermint, wormwood, rose, basil and jojoba oil extracts.

Investigations clearly indicate that the pupal duration of the control was 7.45±0.22 days; this was significantly prolonged only for those previously fed as larvae on leaves treated with garlic (+34.63%), clove (+33.29%) and eucalyptus oil extracts (+23.22%). No significant changes in pupal duration for those previously fed as larvae on leaves treated with peppermint (+16.51), wormwood (+3.36%), rose (+6.44%), basil (+8.46%) and jojoba oil extracts (+1.07%) compared with the control.

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Table 3. Effects of various plant extracts on larvae, pupae of *Tuta absoluta* resulting from treated first larval instar.

Tested plant extract	Larval duration		Pupal weight		Pupal duration	
	Mean ± SD (days)	% of effects	Mean ± SD (Weight / mg)	% of effects	Mean ± SD (Days)	% of effects
Control	11.15 ± 0.39 ^{a}	0	3.43 ± 0.18 ^{a}	0	7.45 ± 0.22 ^{a}	0
Garlic	14.61 ± 1.27 ^{b}	+ 31.03	2.4 ± 0.26 ^{b}	- 30.03	10.03 ± 1.77 ^{b}	+ 34.63
Clove	14.73 ± 1.41 ^{b}	+ 32.1	2.72 ± 0.19 ^{bc}	- 20.7	9.93 ± 1.36 ^{bc}	+ 33.29
Eucalyptus	13.13 ± 1.26 ^{c}	+ 17.76	2.98 ± 0.33 ^{ab}	-13.12	9.18 ± 1.54 ^{bcd}	+ 23.22
Peppermint	12.52 ± 1.44 ^{c}	+12.29	3.05 ± 0.09 ^{ac}	- 11.08	8.68 ± 1.53 ^{ac}	+ 16.51
Wormwood	12.48 ± 1.39 ^{ac}	+ 11.93	3.23 ± 0.24 ^{ac}	- 5.83	7.7 ± 1.52 ^{a}	+ 3.36
Rose	11.58 ± 0.98 ^{ac}	+ 3.86	3.3 ± 0.12 ^{ac}	- 3.79	7.93 ± 1.65 ^{ad}	+ 6.44
Basil	12.25 ± 1.27 ^{ac}	+ 9.87	3.13 ± 0.15 ^{ac}	- 8.75	8.08 ± 1.3 ^{ad}	+ 8.46
Jojoba	12.23 ± 0.31 ^{ac}	+ 9.69	3.28 ± 0.18 ^{ac}	- 4.37	7.53 ± 1.49 ^{a}	+ 1.07
	L.S.D= 1.3		L.S.D= 0.64		L.S.D= 1.25	
	F= 2.13		F= 1.2		F= 6.07	

Means followed by similar letters within the same vertical column are not significantly different at 0.05 level .

- Repellency of various plant extracts to the first larval instar of *T. absoluta*.

The repellency of the tested plant extracts was investigated. Data in table (4) showed that all tested plant extracts had varying degrees of repellent activity. The tested extracts of wormwood, garlic and eucalyptus had strong repellent effect (11.11%, 9.68% and 7.69%, respectively) after 2 hours of treatment showing significant differences compared with other tested plant extracts. However, extracts of jojoba and clove had less repellent activity (-7.14% and -3.45%, respectively).

Increasing the exposure time to 24 hours led to increase the repellent effects in all tested plant extracts where the percentage of repellency ranged from 14.29 % to 37.5%. Garlic had strong repellent effect where repellent percentage reached to 37.5%, this decreased to 33.3, 29.03 and 28.57% with clove, eucalyptus and peppermint oil extracts with no significant effects between them. The repellency was 24.14% with basil with no significant effect compared with peppermint (28.57%). Twenty four hours after application of rose oil extract the repellency was 18.75% compared with 16.13% and 14.29%, for wormwood and jojoba, respectively.

So, it appears that garlic, clove, eucalyptus, peppermint and basil oil extracts had strong repellent effect against 1st larval instar of *T. absoluta*, while jojoba, rose and wormwood oil extracts had less repellent effect against this insect species.

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Table 4. Repellent activity of some plant extracts against the first larval instar of *Tuta absoluta* under laboratory conditions

Tested extracts	Repellency at 2 hours post treatment				Repellency at 24 hours post treatment			
	Mean no. of larvae ± SD (n=50) (Five replicates, 10 larvae for each)							
	Mean no. of larvae settled on		Repellency (R)	% of repellency (PR)	Mean no. of larvae settled on		Repellency (R)	% of repellency (PR)
	untreated disk (C)	treated disk (T)	(C – T) / (C + T)		untreated disk (C)	treated disk (T)	(C – T) / (C + T)	
Garlic	4.25 ± 1.3	3.5 ± 1.1	0.0968 ±0.029 [a]	9.68	5.5 ± 0.5	2.5 ± 0.91	0.375 ±0.12 [a]	37.5
Clove	3.5 ± 0.9	3.75 ± 2.1	- 0.0345 ±0.018 [b]	- 3.45	4.5 ± 0.5	2.25 ± 0.13	0.333±0.034 [ad]	33.3
Eucalyptus	4.5 ± 1.1	4 ± 1.2	0.0588 ± 0.027 [cd]	5.88	5 ± 0.71	2.75 ± 0.4	0.2903 ±0.13 [acd]	29.03
peppermint	3.5 ± 1.5	3 ± 0.71	0.0769 ± 0.029 [ac]	7.69	4.5 ±1.8	2.5 ± 1.6	0.2857 ±0.03 [acd]	28.57
Wormwood	3.75 ± 1.4	3 ± 0.91	0.1111 ± 0.021 [a]	11.11	4.5 ± 0.11	3.25 ± 0.43	0.1613±0.017 [b]	16.13
Rose	3.75 ± 1.5	3.5 ± 1.1	0.0345 ± 0.017 [d]	3.45	4.75 ± 0.83	3.25 ± 0.83	0.1875±0.049 [bc]	18.75
Basil	4 ± 0.71	3.75 ± 0.8	0.0323 ± 0.02 [d]	3.23	4.5 ± 0.5	2.75 ± 0.4	0.2414 ±0.02 [bd]	24.14
Jojoba	3.25 ± 0.11	3.75 ± 1.1	- 0.0714 ±0.018[e]	- 7.14	4 ± 1.2	3 ± 0.71	0.1429 ±0.12 [b]	14.29
	L.S.D = 0.034				L.S.D = 0.12			
	F= 5.82				F= 2.31			

Means followed by similar letters within the same vertical column are not significantly different at 0.05 level .

DISCUSSION

Pest management is facing economic and ecological challenge worldwide due to human and environmental hazards caused by majority of the synthetic pesticides. The present investigations aim to spot light on the ability of some plant extracts to control and to reduce the population of the tomato leaf miner *T. absoluta* so as to serve as an alternative to insecticides. The eight tested plant extracts showed variable toxicity to the insect. According to their potentiality, they can be classified into two groups. The first group is represented by garlic, clove and eucalyptus oil extracts. The second group includes wormwood, peppermint, basil, jojoba and rose oil extracts.

Among the first group, the values of LC_{50} were 680.60, 774.5 and 1008.6 ppm for garlic, clove and eucalyptus oil extracts, respectively while the second group is mildly or moderately toxic and the LC_{50} were 1297.8, 1612.2, 1636.2, 1987.1 and 2527.8 ppm for wormwood, jojoba, peppermint, basil and rose, respectively. Dimetri et al. (1996) found that treatment with neem extracts against *Bemisia tabaci* on tomato reduced its reproduction compared with the control. Many authors evaluated the efficacy of some plant extracts against different insect species (Dos Santos et al. 2010; Regnault-Roger, Vincent & Arnason 2012; El-Wakeil 2013; Ootani et al. 2013; Regnault-Roger 2013; Cavalcanti et al. 2015). Also, Moawad, Ebadah & Mahmoud (2013) recorded that clove, eugenol and isoeugenol caused highly reduction percentage of penetration and accumulative mortality of larvae and caused ovipositional deterrence reaction towards adult stage of *T. absoluta* under laboratory conditions. Hussein et al. (2014) examined the effect of garlic extracts, lemon grass extract, basil, rue, anise and eucalyptus oil extracts, on *T. absoluta* infestation under field conditions; the highest reduction was recorded by garlic extract followed by lemon grass extract and basil oil. Similar data were also reported by Moawad et al. (2015b) who showed that mixed clove, bitter orange and zinc sulfate together had ability to cause highest mortality to *T. absoluta* larvae that reached 97.0%. In this respect, Ebadah, Shalaby & Moawad (2016) revealed that use of clove oil, bitter orange oil and mixture of both (1:1) gave 43.5, 36.9 and 60.6 % reduction of nymphs of white fly count, respectively, while the mortality percentages in larval population of *T. absoluta* were 87.3, 26.3 and 70.5%, respectively.

In an attempt to increase the potency of plant extracts, the commercial formulation of Dipel-2X (*B.t.*) was mixed with the tested extracts. Increase of the larval mortality of *T. absoluta* in varying degrees was reached after treatment with combination of *B.t.* and plant extracts except rose and garlic oil extracts which showed incompatibility with *B.t.* However, the mortality percentage reached to 80% at 2000 ppm in extract of clove + *B.t.*

The results showed that according to the LC_{50} values / ppm, the larvae of *T. absoluta* were more susceptible to clove (308.6) then eucalyptus oil (396.1), wormwood (450), garlic (586), jojoba (708.02), basil (753.6) peppermint (754.9) and finally rose oil extracts (2277).

These findings coincide with those of Salama et al. (1984) using combinations of chemical insecticides and *B.t.* The larvae of *T. absoluta* appeared to be more susceptible to *B.t.* in combination with *Beauveria bassiana* (Torres Gregorio et al. 2009) or to *B.t.* alone (Shehata 2015), and with spinosad (Hashemitassuji et al. 2015).

The results clearly indicate that the biology of *T. absoluta* is obviously affected after treatment with plant extracts. An obvious effect in reducing the growth rate of the larvae was observed especially with clove oil extract (11.04%) in contrast to rose oil extract which affects slightly the growth rate of surviving larvae(2.26%).The larval duration was prolonged after feeding on the tested plant extracts in varying degrees, being significantly retarded with garlic, clove and eucalyptus oil extracts. Accordingly these extracts caused significant reduction in pupal weight and this may be related to low rate of larval feeding. Also, the pupal duration was significantly prolonged for those previously fed as larvae on leaves treated with garlic, clove and eucalyptus oil extracts.

The repellency of the tested plant extracts was evaluated 2 and 24 hours post-treatment. The tested extracts had varying degrees of repellent activity and the extracts of wormwood, garlic and eucalyptus had strong repellent effect (11.11%, 9.68% and 7.69%, respectively) after 2 hours of treatment. Increasing the exposure time to 24 hours led to increase of the repellent effects in all tested plant extracts. Garlic and clove oils had strong repellent effect where repellent percentage reached to 37.5 and 33.3%, respectively, while the repellency decreased to 29.03 and 28.57% with eucalyptus and peppermint oil with no significant effects between them. The percentage of repellency was 24.14% with basil oil, insignificant effect compared peppermint (28.57%).While application of wormwood and jojoba oil had less repellency percentages (16.13% and 12.29%, respectively) with insignificant effects compared with basil and rose oils.

Previous studies showed that the essential oils possess acute contact and fumigant toxicity to different species of insects (Liu and Ho 1999; Abdelgaleil et al. 2009), repellent activity (Wang et al. 2006; Nerio, Olivero-Verbel & Stashenko 2009), antifeedant activity (Huang et al. 1997; Huang, Ho & Kini 1999), as well as development and growth inhibitory activity (Tomova, Waterhose & Doberski 2005; Waliwitiya, Kennedy & Lowenberger 2008). Sabbour & Abd-El-Aziz (2010) reported that mustard and clove oils revealed a strong repellent activity after 7 days (89 and 71%, respectively) against *Bruchidius incarnatus* beetles. While, Zapata & Smagghe (2010) stated that oils extracted from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri* had a very strong repellent activity towards stored-product insect *Tribolium castaneum* .In other studies (Correa 2011; Carlos et al. 2016) showing the potential of use of clove essential oil, whether by contact, repellence or fumigation, the authors observed that clove essential oils can become an alternative to the conventional insecticides for the mortality of populations of *Acanthoscelide obtectus* and *Sitophilus zeamais.* Moawad, El-Behery & Ebadah (2015a) indicated the ability of clove oil against reduction in egg hatchability, life span of larval and adult stage durations of *Galleria mellonella*. The reduction in the population density of *B. tabaci* on tomato plants with neem seed extract (Schmutterer 1988) indicates a repellent effects of this formulation towards adult white fly and this is accordance with Dimetri & Schmidt (1992) who found that bean plant treated with Neem Azal exhibited high degree of deterrence to *Aphis fabae*.

Conclusion:

In conclusion, the tested plant extracts showed to be promising for the control of *T. absoluta* larvae in tomato plants, since it exhibits acute toxicity towards this insect singly or combined with *B.t*. Also, it affects the insect development by reducing its survival, growth rate, prolonging the larval and pupal duration and reducing the pupal weight. Some of these extracts had strong repellent activity.

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