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Efficacy of Three Plant Oil Extracts and their Nano-Emulsions on the Leaf Miner, *Tuta absoluta* Larvae (Meyrick) (Lepidoptera: Gelechiidae)

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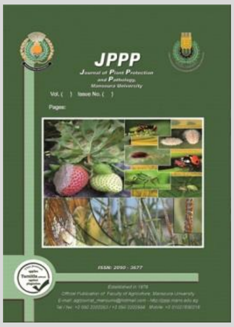

ABSTRACT

In Egypt, tomato crop is the second most important vegetable crop and *Tuta absoluta* comes among the most serious lepidopterous insects that attaching the tomato crop. Due to the dangers of chemical pesticides, nano-emulsions have recently been in pest control programs. The present study looked into the larvicidal activity of the three plant oil extracts and their nano-emulsions against the second instar larvae of *T. absoluta.* Spraying method was used for application on the leaves infested with *T. absoluta* larvae. Results showed that nano-emulsions of essential oils were more effective than traditional essential oils against larvae. The highest mortality proportion caused by the high concentrations of Nano emulsion of essential oils (250 ppm) and essential oils (10000 ppm) under laboratory conditions**.** But the highest reduction percentage in larvae population was caused by essential oil nano-emulsions under field conditions**.** As a conclusion, nanoparticles of essential oil have to be used as bio-pesticides in IPM programs of *T. absoluta*.

Keywords: *Tuta absoluta, Nano-emulsion formulations, Camphor Oil, Marigold Extract, Mandarin Oil.*

INTRODUCTION

The South American tomato pinworm, *Tuta absoluta* (Meyrick) considers as a destructive pest to tomato plants. It is a native to South America, and more recently introduced to Europe and Africa. This pest attacks many nightshade crops, particularly tomato. The larvae cause damage to tomatoes at all stages of development, creating large galleries in their leaves, stems, immature and mature fruits. In tomato crops if no control measures are taken, it can result in yield losses of up to 100%. (Gebremariam, 2015, Desneux *et al*., 2010,). Because of its short generation time, wide host range and its ability to develop insecticide resistance, this insect has become an economical pest. (Biondi *et al*., 2018; Guedes and Picanço, 2012; Tropea Garzia *et al*., 2012). *Bacillus thuringiensis* and *Azadirachta indica* cause a high mortality rate in larvae of *T. absoluta* in both field and semi-field conditions (Hosseinzadeh *et al*., 2019; Hossain, *et al*., 2019; Abd El-Ghany *et al.*, 2018) Due to the dangers of chemical pesticides to the environment and all living organisms, new pest control methods such as nano-emulsions have been recently used against insect pests (Palermo *et al*., 2021, Barik *et al*., 2008). Because nanoparticles have the ability to distribute on large surface areas, they can easily adsorb and bond with other compounds (Gonza'lez-Colomaa *et al*., 2005). Many essential oils contain Alpha-pinene that, derived from pine, piper, lavender, and rosemary. Essential oils have been reported as repellents and antifeedants against *Spodoptera litura* (Fan *et al*., 2011). For example, *Calendula officinalis* L. flowers contain active chemical components, essential oils, saponins, flavonoids, terpenoids, carbohydrates, fatty acids, amino acids, lipids, carotenoids, and quinones, (Preethi *et al.*, 2006, Bilia *et al*., 2002), and citrus essential oils contain alcohols, terpenes, aldehydes, sesquiterpenes, sterols and esters. Marouf (2020) discovered that nano chitosan, followed by mandarin crust oil, was the most effective against *Spodoptera littoralis* larvae, and caused morphological changes in the treated larvae.In addition, Chen *et.al.* (2013) found that Camphor (*Cinnamomum camphora*. L.) has antimicrobial, anticoccidial, antiviral, antinociceptive, anticancer, and insecticidal properties against many pests. As well, citrus peel oils have insecticidal, antitumor, antioxidative, antimicrobial, and antifeedant activity against several pests (Abdelgalei *et al*., 2009; Kostic, *et al*., 2008; Dayan, *et al*., 2009; Gonza´lez-Colomaa *et al.* 2005). Thus, the present research aimed to examine the insecticidal activity of three plant extracts and their nano-emulsions, against *Tuta absoluta* larvae.

MATERIALS AND METHODS

Insect culture

Tomato leaves infested with *T. absoluta,* were obtained from untreated farm at the experimental farm of Mansoura University. The larvae had been reared for two generations. The leaves were provided daily in the laboratory setting at 25 ± 2 ºC, photoperiod of 14 hours light and 10 hours dark, and RH 60–70 percent. Adults were kept separately and mated in clean jars (4 lb.) on the third day of emergence. Adults were fed at 10 % honey solution and fresh untreated leaves of tomato were provided for egg laying. After hatching, were daily provided with fresh untreated leaves of tomato. The larvae had been reared for two generations and the 2nd instar larvae of the *T. absoluta* were used for application (Bajonero and Parra,2017).

Plant materials and essential oils extraction

Essential oils

The mandarin and camphor essential oils were extracted from peels of mandarin and leaves of camphor

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plant, respectively. Steam distillation was used to extract volatile oils at Plant Protection Research Institute, Agricultural Research Center, Mansoura, Egypt. Following separation and drying over anhydrous sodium sulphate the oil were stored in dark glass bottles at 4° C. With respect to marigold (*Calendula officinalis* L.) extraction, at room temperature, flowers of marigold were dried about four weeks and ground into powder, the powder was soaked in a flask, with an equal parts hexane, acetone, and ethanol (1:1:1) for seven days. The flask was shaken and filtered. The extract was pressure evaporated, weighed, and stored in a freezer until used (Marouf, 2022). The extract was dissolved in water to preparing the tested concentrations. Tween-80 emulsifier was added 0.01 percent.

To prepare the tested concentrations, essential oils were dissolved in water, and 0.01 percent Tween-80 emulsifier was added to the dilution.

Nano emulsions of plant extracts.

Nano-emulsions were created according to Youssef *et al*. (2018) and Jerobin *et al*. (2012). Essential oils were combined with distilled water (at a ratio of 1:2) after that Tween 80(2 percent) was added at a rate of 1 milliliter/liter. The emulsions were sonicated for half - hour at 60 Hz with an ultra-sonic cleaner set, version WUC-DO3H 290W. A high-power ultrasonication probe was used to ultrasonically treat the coarse emulsion for 1 minutes with model VCX750, 750W, 20 kHz. After another half-hour on the sonicator, the samples were kept at 4 degrees Celsius for bioassays. Five concentrations of essential oils (10000, 5000, 1000, 500, and 100 ppm) and their nano- emulsions (250, 100, 80, 40, and 20 ppm) were prepared.

The (w/v) of (tested material and distilled water) was used to calculate the concentrations of each material. Each material was tested in five different concentrations, the LC-P lines created using three replicates for each concentration.

To analysis of gas chromatography-mass spectrometry. GC- MS analysis of essential oils was performed according to the Marouf (2022).

Laboratory experiments:

The spray method was applied on the leaves infested with 2nd instar larvae of *T. absoluta*. There were five concentrations and three replicates for each concentration. The mortality line was estimated using 10 *T. absoluta* larvae per replicate. The leaves were sprayed with various concentrations. The five concentrations of essential oils (10000, 5000, 1000, 500, and 100 ppm) and their nano-emulssions (250, 100, 80, 40, and 20 ppm) were tested. The percentages of mortality after one, three, five, and seven days were recorded.

According to Abbott (1925), mortality rates were calculated and adjusted. LC50 values are calculated using a statistical method of probit analysis (Finney, 1971). The LC50 index was calculated using Sun (1950) equation.

$$\textbf{LC}_{50}\ \textbf{Toxicity index} = \frac{\textbf{LC}_{50}\ \textbf{of the highest effective compound}}{\textbf{LC}_{50}\ \textbf{of the lowest effective compound}} \times 100$$

Field experiments:

This experiment used LC90 for each material of nano essential oils. Tomato plants were subjected to a field experiment in Aga -Abo Dawood region, El-Dakahlia, Egypt. Cultural practices for tomato plants were carried out in accordance with Egyptian Ministry of Agriculture guidelines. Four replicates and the design of complete randomized block was applied as follows.

Running water as a control treatment – in addition to LC90 nano -emulsions of (mandarin oil, camphor oil and marigold extract were sprayed against *T. absoluta* which infested the selected tomato field.

After 5, 7, and 10 days of spraying with the treatments, the percentage reduction in larval infestation was calculated using the Henderson and Tilton formula (1955) as follows:

$$\textbf{Reduced mortality (\%)} = [1 - (C_b / C_a \times T_a / T_b)] \times 100$$

Where:

C_b= The number of alive pest individuals before treatment under control.
C_a= The number of alive pest individuals after treatment under control.
T_a= The number of pest individuals still alive after treatment.
T_b= The number of pest individuals still alive before treatment.

RESULTS AND DISCUSSION

1. Laboratory Experiments:

The effect of essential oils and their nano-emulsions against *T. absoluta* larvae:

The effect of plant oils and their nano-emulsions on *T. absoluta* larvae was described and demonstrated in Table1(A, B), Table 2 (A, B), as well as Fig.1. (A, B). When compared to untreated treatments, the impact of different concentrations of tested treatments on *T. absoluta*, resulted in a high mortality proportion (Table1(A, B)). The rate of mortality increased as concentration increased, the findings revealed that nano-emulsion of essential oils are more effective than essential oils. The highest mortality rates (96.67%, 96.66 % and 86.67%) were caused by nano-emulsion of essential oils (of mandarin, marigold, and camphor), respectively at high concentration (Table 1(A)), followed by (93.33 percent, 80 percent and. 76.67 percent) mortality rates at high concentration of essential oils of (mandarin, marigold, and camphor) respectively (Table 1(B)).

The nano- emulsion of mandarin was the most effective against *T. absoluta* larvae followed by nano-emulsion of marigold and camphor, which had toxicity indexes 100, 67.55 and 17.77 percent, the LC50 values were 5.021, 7.433, and 28.25 ppm, and the LC90 values were 116.26, 145.12 and 794.49 for nano- emulsion oils of mandarin, marigold, and camphor, respectively. (Table 2A). On the contrary, essential oil of marigold was the highest effective treatment to *T. absoluta* followed by essential oils of mandarin and camphor, (the LC50 values were 309.73, 784.87 and 872.71 ppm, in addition, the LC90 values were 45446, 20552.9 and 75172.98 and the toxicity index were 100, 39.46, and 35.49 %) for the essential oils of marigold, mandarin and camphor, respectively. Table 2 (B).

The impact and malformation of *Tuta absoluta* larvae after treatment with essential oils and their nano-emulsions

Figure 2 summaries and illustrates the morphogenetic effects. Treatments resulted in certain malformation in *T. absoluta* larvae treated by nano- emulsion of mandarin essential oil Fig.2A1and mandarin essential oil Fig.2A2, compared to the Control Fig. 2 A3, and nano emulsion of marigold essential oil Fg.2B1, marigold essential oil Fg.2B2, and the Control Fg.2B3, and nano emulsion of Camphor essential oil Fig.2 C1, Camphor essential oil Fig.2C2, and Control Fig.2C3, respectively. As a result, all treatments are showing an obvious effect on the larvae of *T. absoluta* and cause malnutrition.

Field experiments

The reduction % in larval population of *T. absoluta* after five, seven and ten days illustrates and summaries in Tables (3 and 4). According to the findings in Table (3) the total reduction caused by nano- emulsion of mandarin crust oil was greater after seven days compared to other days. It was recorded 96.88%. followed by 96.36, 83.86% after seven, ten and five days, respectively. On the other hand, the total reduction caused by nano- emulsion of marigold ext. and camphor oil were higher after ten days compared to other days. It was recorded 77.15, 85.88 and 91.76% (for marigold extract) and recorded 80.91, 88.99 and 94.09% (for camphor oil) after five, seven, ten days, respectively.

According to Table (4) the average total reduction % of nano- emulsion of mandarin crust oil, marigold extract, and camphor oil were 92.36, 84.93 and 87.99 %, respectively.

Table 1. Mortality % of essential oils (B) and their nano-emulsions (A) *against Tuta absoluta* larvae under laboratory conditions of 27±2 ºC and 65±5% RH.

(A)			(B)		
Nano Emulsion of essential oils	Conc. (ppm)	Total Mortality %	Essential oils	Conc. (ppm)	Total Mortality %
	10	63.33		100	30
	40	76.67		500	36.67
mandarin crust oil	80	86.67	mandarin crust oil	1000	43.33
	100	90		5000	73.33
	250	96.67		10000	93.33
	10	60		100	33.33
	40	70		500	60
Marigold Ext.	80	80	Marigold Ext.	1000	66.67
	100	90		5000	73.33
	250	96.66		10000	80
	10	40		100	30
	40	50		500	36.67
camphor oil	80	60	camphor oil	1000	53.33
	100	66.67		5000	70
	250	86.67		10000	76.67
Control (Distilled water)	----	0.0	Control (Distilled water)	----	0.0

Table 2. The toxic effect of essential oils (B) as well as nano-emulsions (A) *on Tuta absoluta* larvae.

(A)

Nano Emulsion of essential oils	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90} / LC_{50}	R	P
	10	63.33							
	40	76.67							
mandarin crust oil	80	86.67	5.0211	116.26	0.9392± 0.2529	100	23.1542	0.9789	0.9478
	100	90							
	250	96.67							
	10	60							
	40	70							
Marigold Ext.	80	80	7.433	145.12	0.9931±0.2569	67.55	19.5237	0.9383	0.5359
	100	90							
	250	96.67							
	10	40							
	40	50							
camphor oil	80	60	28.25	794.49	0.8844±0.2341	17.77	28.1235	0.9351	0.5596
	100	66.67							
	250	86.67							

R: Regression **P: Propability**

(B)

essential oils	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity indexLC_{50}	LC_{90} / LC_{50}	R	P
	100	33.33							
	500	60							
Marigold Ext.	1000	66.67	309.73	45446	0.5915±0.1540	100	146.727	0.9652	0.7669
	5000	73.33							
	10000	80							
	100	30							
	500	36.67							
mandarin crust oil	1000	43.33	784.87	20552.9	0.9038± 0.1635	39.46	26.186	0.9235	0.145
	5000	73.33							
	10000	93.33							
	100	30							
	500	36.67							
camphor oil	1000	53.33	872..71	75172.98	0.6623±0.1537	35.49	86.137	0.9802	0.844
	5000	70							
	10000	76.67							

R: Regression **P: Propability**

Table 3. Effect of essential oil nano- emulsion on *T. absoluta* infestation percentages under field conditions after, five, seven and ten days (estimated as reduction percentages).

Nano Emulsion of essential oils	LC_{90} ppm.	1st replicate Red.%	2nd replicate Red.%	3rd replicate Red.%	4th replicate Red.%	Total Mean reduction %
			After five days			
mandarin crust oil	116.26	85	82.5	85.71	82.22	83.86
Marigold Ext.	145.12	70	85.42	78.57	74.6	77.15
camphor oil	794.49	87.5	82.5	71.43	82.22	80.91
			After seven days			
mandarin crust oil	116.26	100	100	87.5	100	96.88
Marigold Ext.	145.12	86.67	87.04	81.25	88.57	85.88
camphor oil	794.49	100	84.44	87.5	84	88.99
			After ten days			
mandarin crust oil	116.26	100	100	100	85.45	96.36
Marigold Ext.	145.12	89.09	88.33	100	89.61	91.76
camphor oil	794.49	90.91	100	100	85.45	94.09

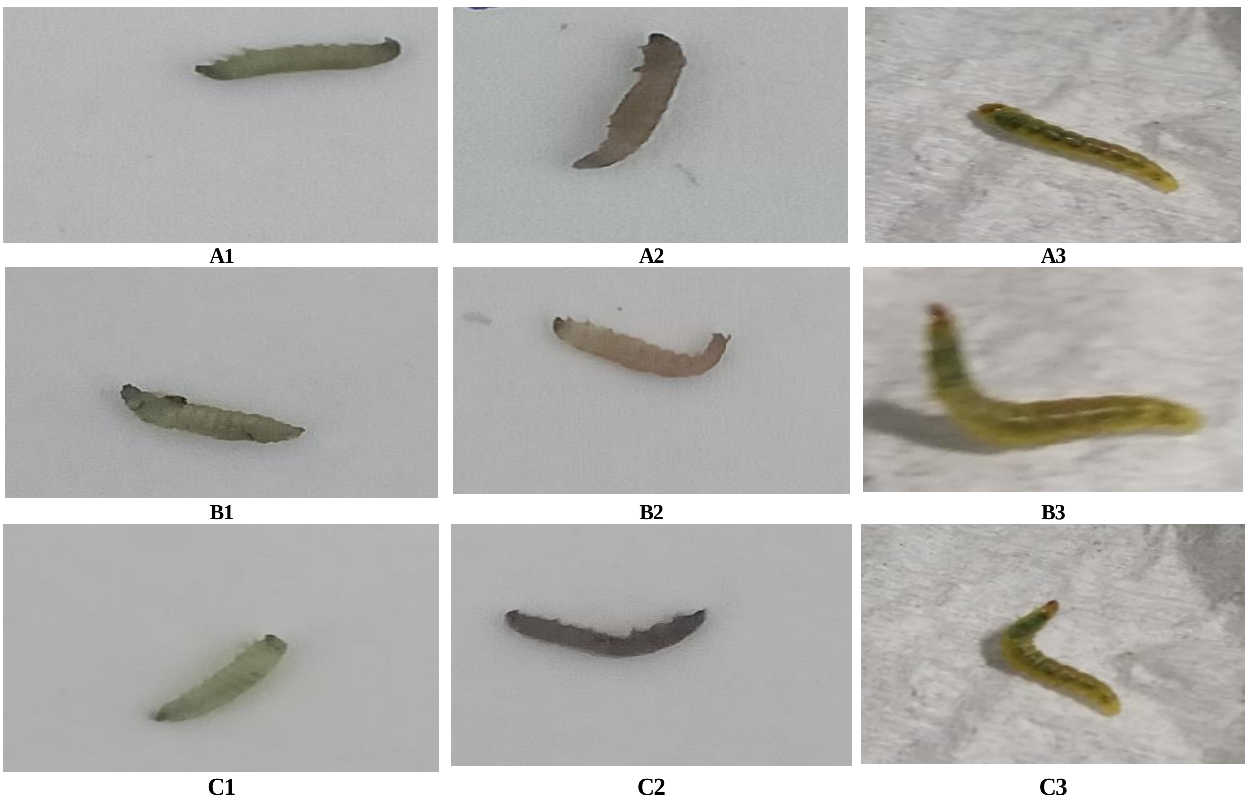

Fig. 2. *Tuta absoluta* larvae malformation treated with nano-emulsions of essential oils of (mandarin, marigold and camphor) (A1, B1,C1), essential oils of (mandarin, marigold and camphor) (A2,B2,C2) and control (A3, B3,C3) respectively.

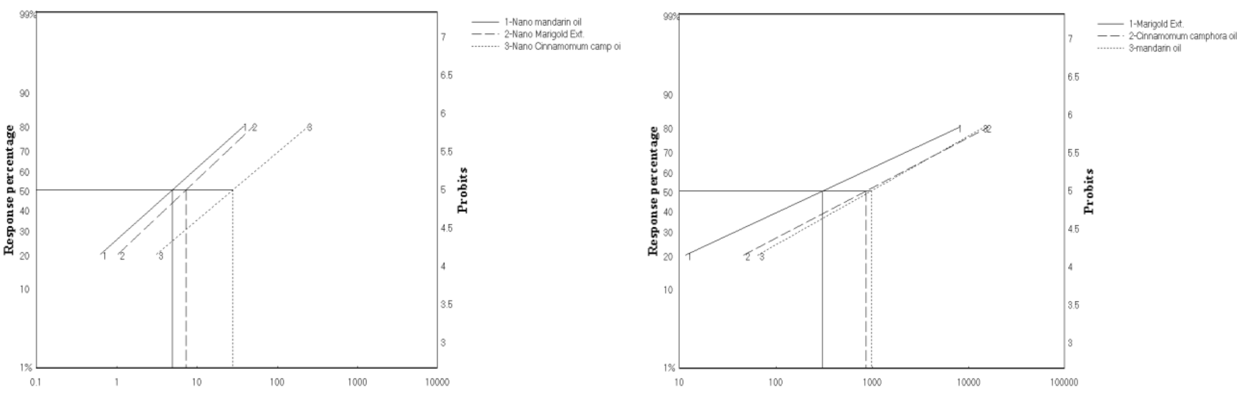

Fig. 1. LC-P lines of essential oils (B) as well as nano-emulsions (A) *on Tuta absoluta Larvae*.

Table 4. Mean effect of essential oil nano- emulsion on *T. absoluta* under field conditions (estimated as reduction percentages).

Nano Emulsion of essential oils	LC_{90} ppm.	1st scan average reduction	2nd scan average reduction	3rd scan average reduction	Average of total reduction
mandarin crust oil	116.26	83.86	96.88	96.36	92.36
Marigold Ext.	145.12	77.15	85.88	91.76	84.93
camphor oil	794.49	80.91	88.99	94.09	87.99

Discussion

The larval of *T. absoluta* feed on all parts of the plant, except roots, which resulting in significant crop losses that reached up to 100 % on tomato plant (Biondi *et al.*, 2018; Desneux *et al*., 2010). The use of chemical pesticides is costly, ineffective, and potentially harmful to the environment. Alternatives that are more environmentally friendly, bio-based, and long-term must be used to mitigate this. Natural ingredients, such as insecticidal plant extracts, can be used for successful pest control (El Hajj *et al*., 2017; Tarusikirwa *et al*., 2020). The results showed that nano-emulsions of plant oil extracts were more toxic to *T. absoluta* larvae than essential oils. It had the highest insecticidal activity against *T. absoluta* larvae. There were high mortality proportions (96.67, 96.66, and 86.67 %) at high concentrations under laboratory conditions, in addition, the relatively high mean reduction percentages (92.36, 84.93 and 87.99 %) under field conditions of nano-emulsion of (mandarin, marigold and camphor). These results are consistent with Ahmed *et al.* (2020), who reported that aniseed oil nano-emulsion are more toxic to *Tribolium castaneum* than essential oil. Five new pesticides were evaluated on *T. absoluta* on tomato plants (Barakat *et al.* 2015). The impacts of pesticides on egg and larvae were calculated after two, five, and seven days of application. All pesticides had the highest percentage of larvae reduction after seven days. Our results indicated that, the total reduction caused by nano- emulsion of mandarin crust oil was greater after seven days compared to other days. On the other hand, the average total reduction caused by nano-emulsion of marigold, and camphor were greater after ten days compared to other days. Our findings agreed with those of Dimetry *et al.* (2019), who discovered that nano-emulsions of camphor, thyme, peppermint, and sage oils more toxic than the essential oils against the *Agrotis ipsilon* larvae. According to Mohanny *et al.* (2020), the effect of five pesticides increased and peaked after 72 hours in the bioassay. The toxicity curve for emamectin benzoate was the steepest, and the curve for imidacloprid was the flattest; however, fipronil and indoxacarb are in the middle. Also, Braham *et al.* (2012b) and Bala *et al.* (2019), demonstrated that emamectin benzoate was effective against *T. absoluta* populations in the laboratory. The main constituents of *Citrus reticulate* oils were terpinene, (E)-ocimene, Limonene, -myrcene, linalool, -Pinene and sabinene (Lawal *et al*., 2014). The findings are in agreement with those of a recent study by Marouf (2020), since D-Limonene was the most abundant compound in mandarin oil, accounting for 32.38 percent of the total compound. According to Marouf (2022), GC-MS analysis revealed that 6-epi-shyobunol (Longiborneol) is the major constituents of marigold extract, accounting for 44.93 % of total compounds. The identification of 34 different compounds in marigold extract led to the discovery of 74.36 % of the total extract. The combination of plant essential oils and nano-emulsions is one of the most effective tools for developing environmentally friendly control in the field that be used in integrated pest management. Jallow *et al.* (2018) discovered that various bio-pesticide formulations (*B. thuringiensis*, *B. bassiana*, and neem) influenced on *T. absoluta* larvae in the lab, in field experiments, showed highly significant pest control, which was consistent with the findings of Gonzalez-Cabrera *et al*. (2011), Pires *et al* (2010) and Rodriguez *et al*. (2006). Furthermore, the bioassay findings experiment revealed that different bio-pesticides were highly effective against larvae of *T. absoluta*.

CONCLUSION

The toxicity of plant extracts and their nano-emulsions against *T. absoluta* larvae revealed that nano-emulsions are an important pest control alternative to chemical pesticides

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فعالية مستخلصات الزيوت النباتية الثلاثة ومستحلباتها النانونية على يرقات حافرة الأوراق توتا ابسليوتا

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الملخص

في مصر، يعتبر محصول الطماطم ثاني أهم محصول نباتي، وتأتى*Tuta Absoluta* (Meyrick) (Lepidoptera: Gelechiidae) من بين أخطر الحشرات حرشفية الأجنحة التي ترتبط بمحصول الطماطم. نظرًا لمخاطر المبيدات الكيميائية، فقد تم مؤخرًا استخدام مستحلبات النانو لمكافحة الآفات. تناولت الدراسة الحالية فعالية المستخلصات النباتية الثلاثة ومستحلباتها النانوية ضد يرقات الطور الثانى لحشرة التوتا ابسليوتا. تم استخدام طريقة الرش للتطبيق على الأوراق المصابة بيرقات التوتا ابسليوتا واظهرت النتائج ان مستحلب النانو للزيوت المختبرة أكثر فعالية من الزيوت النباتية التقليدية ضد اليرقات وتسبب أعلى نسبة موت عند التركيزات العالية لمستحلب النانو من الزيوت النباتية (250 جزء في المليون) والزيوت النباتية (10000 جزء في المليون) تحت ظروف المعمل. لكن اعلى نسبة انخفاض في تعداد يرقات التوتا ابسليوتا كانت بسبب مستحلبات النانو من الزيوت النباتية في ظل الظروف الحقلية. الخلاصة: يجب استخدام الجسيمات النانوية من الزيت العطرى كمبيدات حشرية حيوية في برامج المكافحة المتكاملة للآفات الخاصة ب التوتا ابسليوتا.