Influence of insect traps and insecticides sequential application as a tactic for management of tomato leafminer, *Tuta absoluta* (Meyrick), (Lepidoptera: Gelechiidae)

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**Abstract**

**Background:** Tomato crop, *Lycopersicon esculentum* L. (Solanaceae), usually attacked by many insect species, including the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), where its larvae cause damage up to 80–100% by due to its feeding habits by making tunnels in all tomato parts. The influence of insect traps application and insecticides sequential as a tactic for *T. absoluta* management was carried out.

**Results:** Sex pheromone and sticky traps were used for mass trapping this pest; sex pheromone traps were more effective than the sticky one; the number of caught insects was higher in the untreated plots than the treated one. Also, the general mean number of captured insects by sex pheromone traps was 432.89 and 633.40 (vegetative stage) and 691.3 and 1865.5 (fruiting stage) adults/trap/week, for treatment and control, respectively. When the first infestation appeared, the non-conventional insecticides were consecutively sprayed once/week for 3 weeks with the following order: Radiant, Coragen, and Emperor (during the vegetative stage). After that, the formulated essential oils Nimbecidine (commercial) and Rosa (prepared) were respectively sprayed twice/week for 2 weeks (during the fruiting stage). The general average of reduction in infestation reached 95.81% when non-conventional insecticides were used, while it reached 92.15% when the formulated essential oils were used. Finally, at the end of the experiment, promising mean reduction in infestation reached 93.98% when non-conventional insecticides were used.

**Conclusions:** The results indicated that insect traps and insecticides sequential application as a tactic for management *T. absoluta* were more effective; the rotation of insecticides avoid build up resistance and achieved satisfied reduction (93.98%) in the infestation and high yield production.

**Keywords:** *Tuta absoluta*, Sequential application, Insect traps, Non-conventional insecticides, Formulated essential oils
**Introduction**

Tomato plants (*Lycopersicon esculentum* L.), which belong to family Solanaceae, is an important and profitable vegetables for fresh marketing processing. It is the 6th most valuable cultivated plant. In Africa, total production is nearly 37.8 million tons annually; Egypt, Nigeria, Tunisia, and Morocco were the greatest producers. Tomato plants usually attacked by a great variety of insects especially tomato leafminer, *Tuta absoluta* (Meyrick 1917) (FAOSTAT 2017; Kushwaha et al. 2018). *T. absoluta* was firstly recorded in both Algeria, Morocco, and Libya in 2008 and 2009 and continued to invade Egypt in 2010 (Moussa et al. 2013; Salem and Abdel-Moniem 2015; Sylla et al. 2017; Biondi et al. 2018). The larvae of this insect have destructive effects on tomatoes and causing yield losses being 80–100%. *T. absoluta* is difficultly manageable due to two reasons: larvae feeding habits and insects ability to build up resistance. Such resistance enforces the farmer to aggravatingly and repeatedly spray chemical insecticides; hence, the insect becomes highly resistant towards synthetic insecticide and shows the reduced effectiveness of control (Abd El-Salam et al. 2015; Ayalew 2015; Tonnang et al. 2015; Chidege et al. 2017; Aynalem 2018; Ndor 2018; Sammour et al. 2018).

Management of *T. absoluta* becomes problematic, especially when the infestation level is high, as well as the failure of synthetic pesticides application for controlling this pest because of development of insect resistance, high operating costs, and the environmental contamination. Therefore, we need to develop alternative solutions. Application of natural compounds, such as plant essential oils, is considered as an alternative to chemical pesticides because of their lower toxicity against non-target organisms and low persistence in the environment (Chegini and Abbasipour 2017; Birhan 2018).

The decision scheme of insecticides which used for managing of *T. absoluta* is largely based on adult captures with sexual pheromone traps, which correlated of both larval damages and yield losses. Using chemical insecticides in combination with other tactics such as natural formulations and mass trapping with sex pheromones as integrated pest management (IPM) is the most useful control method, where it controlled the *T. absoluta* and increased the yield production and the quality of the marketable tomato fruits (Miranda et al. 2005; Benvenga et al. 2007; Taha et al. 2013; Giorgini et al. 2018; Abd El-Salam et al. 2019).

The purpose of the present study is to investigate certain IPM tactic in the management of *T. absoluta*, such as sex pheromone and sticky traps for monitoring and mass trapping the insects, associated with selective non-conventional insecticides (during vegetative stage) and formulated essential oils (during fruiting stage) as well as estimate the tomato yield production.

**Materials and methods**

From our preliminary studies (laboratory and field), some commercial and natural formulations with different modes of action were evaluated against the 2nd larval instar of *Tuta absoluta*. The results revealed that the most effective compounds were Emperor 0.5% EC (avermectins, Shandong Jingbo Agrochemical Co., China), Coragen 20% SC (diamide, Dupont, Canada), and Radiant 12% SC (spinosyns, Dow Agrosciences, England) as non-conventional insecticides as well as the natural compounds Rosa 5% EC (prepared formulation of *Rosmarinus officinalis*) and Nimbecidine 3.5% EC (commercial formulation of Azadirachtin), so, they have been selected for the sequential application against *T. absoluta*, using the LC90 values (Table 1) (Abdelmaksoud 2019).

**Field experiments**

Field experiments were carried out on infested tomato plants (Super strain B) with *T. absoluta* cultivated at Elflash village, BaniSweif Governorate, Egypt, to investigate the efficiency of insect traps associated with chemical insecticides or natural compounds. A known area (1/4 fed.) was divided into six plots including three replicates (for treatment and control); each one was 175 m².

**Mass trapping of *T. absoluta* adults**

To start the process of sequential application of selected treatments, sex pheromone and sticky traps were used for monitoring and mass trapping these insects. The survey study was carried out from the cultivation beginning (1st week of April) to the end of the season (3rd week of July). The average of temperature and humidity were recorded.

**Sex pheromone traps**

A density of 20 traps/fed. was conducted under open field conditions. Plastic basins with 40 × 30 × 15 cm were used as water pan traps filling with water and baieted with the synthetic sex pheromone. Traps were ranked on the tops of 20 cm wood sticks and placed just above the plant canopy. Pheromone capsules were renewed at 2 weeks interval. The level of water in the traps was adjusted when it was necessary. All adults captured were counted and recorded weekly to monitor adult populations and to determine the suitable time for starting using insecticides.

**Sticky traps**

Paper board traps (22.5 × 35 cm.) were used. Traps were coated on one face with a thin layer of insect viscous
The sticky traps were fastened vertically to stakes just above the plant canopy in a randomized pattern. Trapping surface was faced towards the east. The traps were applied at 24 traps/fed. *T. absoluta* moths on each trap surface were counted, and their monitoring was performed on regular weekly basis intervals.

### Insecticides sequential application

When the first infestation appears during the vegetative stage, the most efficient commercial insecticides (Radiant, Coragen, and Emperor) were sprayed by three folds of LC90 values successively once/week for 3 weeks. Firstly, Radiant followed by Coragen then Emperor. While during the fruiting stage, the most promising natural oil-based formulations (Nimbecidine and Rosa) were sprayed successively twice/week for 2 weeks, first Nimbecidine then Rosa (Table 2 and Fig. 1). The levels of infestation were recorded. Control plants were sprayed with an equal volume of water only.

Leaves of five plants (10 leaves/plant) of treated and untreated plots were collected randomly and transferred in paper bags to the laboratory. Samples were taken before spray and after treatment twice a week (3 and 7 days from each week) for examining and counting of alive larvae. The mortality percentages of *T. absoluta* larvae were determined at each collection interval. The percent reduction of infestations was calculated according to Henderson-Tilton formula (Henderson and Tilton 1955).

### Yield production

At the harvest, yield of marketable ripe fruits was recorded and expressed as ton/fed. All fruits harvested (about five successive harvest) from each plot along the harvesting period were weighted to calculate the total yield per fed. (ton/fed.), as well as the total cost, and the net profits for fed. and for ton of tomato yield were also calculated. The income statement was used to estimate the profit of tomato production.

### Increase of yield (%)

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\text{Increase of yield (\%) } = \frac{\text{Yield of treated tomato} - \text{Yield of untreated tomato}}{\text{Yield of untreated tomato}} \times 100
\]

### Cost of treatments

Cost of various insecticides was taken as per market price, and labor charges were calculated. The total cost of spraying was calculated on the basis labor requirement per fed./day. Market price of insecticides and labor charges were summed up to work out the total cost of application of each treatment. This cost was taken as an additional cost required for treatment against the tested insect.

### Additional income over control

Additional income over control was calculated by multiplying the additional yield over untreated control with prevailing average local market price of tomato fruits.

### Net profit

This was calculated by subtracting the additional cost required for treatment from the monetary benefit for each treatment.

### Cost benefit ratio

The cost benefit ratio (CBR) was calculated by dividing the net monetary return by total additional cost due to treatment.

### Statistical analysis

The insecticide efficacy data were subjected to Probit analysis to obtain the LC90 values using the Statistical Package for the Social Sciences (SPSS) 25.0 software program (SPSS 2017). The values of LC90 were considered significantly different if the 95% confidence limits did not overlap.
**Results**

**Mass trapping of *T. absoluta* adults**

**Sex pheromone traps**

The data set out in Table 3 indicated that the mean numbers of the adult caught in the first inspection before begging the insecticides sequence applications were 263 and 241 adults/trap/week for sequential treatment and control, respectively, then increased gradually with time elapsed. The general mean number of captured insects was 432.89 and 633.40 (vegetative stage) and 691.30 and 1865.50 (fruiting stage) adults/trap/week, respectively, where the average of temperature and humidity was 26.2 °C and 39.1% (vegetative stage) and 28.6 °C and 34.5% (fruiting stage), respectively.

This increase in the number of caught insects at the end of the season in spite of using the program of insecticides sequence may be due to the attractant of the sex pheromone from the neighboring farms since these experiments were not conducted under control conditions (open field). The success of this trap partially related with the pesticide sequences; however, if the situation was under control, it will be possible to emphasize the role of the trap with the IPM program.

**Sticky traps**

The data in Table 3 showed that in the first inspection, the mean catches of *T. absoluta* at the begging of the experiment were 8.4 and 8.0 adults/trap/week for treated and untreated plots, respectively. While the mean number of captured insect drastically increase at the end of the season until reached 29.8 and 31.5 adults/trap/week for treated and untreated plots, respectively.

In view of the above results, it is clear that sex pheromone traps captured significantly more moths than sticky traps.

**Insecticides sequential application**

In this program, from our previously results, the tested chemical insecticides which showed high efficiency in reducing insect counts under field conditions were consecutively sprayed with the following order: Radiant,
Table 3 Total count of *T. absoluta* moths collected by the sex pheromone and sticky traps associated with sequential applications during cultivated season

| Month | Inspection time (week) | Mean no. captured adults/trap | Sex pheromone traps | Sticky traps | Temp. average (°C) | Humidity average (%) |
|-------|------------------------|-------------------------------|---------------------|--------------|--------------------|---------------------|
|       |                        | Treatment ± (SD)             | Control ± (SD)      | Treatment ± (SD) | Control ± (SD) |                    |
|       |                        | Vegetative stage             | Fruiting stage      |              |                    |                     |
|       |                        | Treatment ± (SD)             | Control ± (SD)      |              |                    |                     |
| April | 3rd week               | 263 ± 16                     | 241 ± 20            | 8.4 ± 2.3    | 8.0 ± 1.7         | 24.5                | 44.4                |
|       | 4th week               | 259 ± 19                     | 268 ± 17            | 7.4 ± 1.7    | 9.0 ± 3.3         | 26.8                | 38.3                |
|       | Mean                   | 261 ± 17.5                   | 254.5 ± 18.5        | 7.9 ± 2.0    | 8.5 ± 2.5         | 25.7                | 41.4                |
| May   | 1st week               | 312 ± 11                     | 327 ± 15            | 10.6 ± 3.0   | 15.0 ± 2.7        | 24.8                | 43.0                |
|       | 2nd week               | 463 ± 31                     | 583 ± 24            | 11.0 ± 2.3   | 17.5 ± 3.7        | 24.4                | 43.7                |
|       | 3rd week               | 397 ± 27                     | 533 ± 29            | 11.4 ± 3.7   | 18.0 ± 3.0        | 26.5                | 39.2                |
|       | 4th week               | 436 ± 34                     | 567 ± 31            | 15.8 ± 2.0   | 22.5 ± 2.0        | 25.6                | 37.5                |
|       | Mean                   | 402 ± 25.8                   | 502.5 ± 24.8        | 12.2 ± 2.8   | 18.25 ± 2.9       | 25.3                | 40.9                |
| June  | 1st week               | 588 ± 22                     | 873 ± 17            | 15.8 ± 3.3   | 26.0 ± 3.0        | 27.6                | 35.6                |
|       | 2nd week               | 547 ± 36                     | 963 ± 34            | 14.2 ± 2.7   | 30.0 ± 2.0        | 28.4                | 34.2                |
|       | 3rd week               | 631 ± 29                     | 1346 ± 42           | 17.0 ± 3.7   | 33.5 ± 3.3        | 27.4                | 35.9                |
|       | Mean                   | 432.89 ± 25                  | 633.40 ± 25.4       | 12.40 ± 2.7  | 19.94 ± 2.7       | 26.20               | 39.10               |
|       | General mean           | 691.3 ± 26.8                 | 1865.5 ± 31.8       | 24.10 ± 3.1  | 34.08 ± 2.8       | 28.6                | 34.5                |

Fig. 1 Sequence of insecticides and the naturally formulations throughout the season

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Coragen, and Emperor (during the vegetative stage). After that, the tested naturally based formulated compounds Nimbecidine and Rosa were respectively sprayed (during the fruit stage) (Table 2 and Fig. 1), as well as insect traps. This integration will be mandatory to minimize build up resistance in *T. absoluta* populations and increase the efficiency of pest control.

Results revealed that the mentioned sequences achieved considerable reduction percentages in tomato plants infested by *T. absoluta* (Table 4). The mean percent reduction in infestation after three successive spraying during 3 weeks (one spray/week for each insecticide) was 91.61% for Radiant, 99.38% for Coragen, and 96.45% for Emperor. Concerning treatment with naturally based formulated compounds, four successive spraying during 2 weeks were applied (2 sprays/week for each formulation). The mean percent reduction in infestation reached 93.67% for Nimbecidine and 90.63% for Rosa (Table 5).

In conclusion, the general average of reduction in infestation reached 95.81% when non-traditional chemical insecticides were used during the vegetative stage, while it reached 92.15% when naturally based formulated compounds were used during the fruit stage. Finally, at the end of the experiment, we achieved the promising mean reduction in infestation reached 93.98%.

**Yield production**
The data of tomato yield as influenced by various treatments are presented in Table 6. It could be seen from the table that treatments gave significantly higher yield over untreated control. The tomato yield reached 28.25 t/fed. when compared with control (8.4 t/fed.), which gain 19.94 t/fed. and 238.32% more than the untreated control.

It is seen from Table 6 that treatments achieved the maximum net monetary returns of 31647 EG pound per fed. and 1120.25 EG lb/t, in comparison to untreated control which implemented 6146 EG lb/fed. and 736.05 EG lb/t. Also, treated plants achieved the highest cost benefit ratio of 1:7.6. The treated plots recorded maximum gross income; it was 42093 EG lb/fed. compared with untreated control (12442 EG lb/fed.).

In conclusion, treatments represent a promising effect in controlling the pests as well as gain a net profit as compared with the control.

**Discussions**
Complementary strategies of pest management aim to diminish the use of insecticides through an efficient monitoring of pest population in order to choose the correct time of pesticide application. Lepidoptera pheromones have been successfully used for insect monitoring and mating disruption of insects (Taha et al. 2013; Brah- ham 2014; Giorgini et al. 2018).

**Table 4** Effect of sequential application of certain commercial non-traditional chemical insecticides on *Tuta absoluta* applied three times (during vegetative stage)

| Spray number | Day after treatment | Control | Radiant | Coragen | Emperor |
|--------------|---------------------|---------|---------|---------|---------|
|              | Alive | Alive | R% | Alive | Alive | R% | Alive | Alive | R% |
| B-spray Zero | 6.67 | 7.33 | 0.00 | 7.33 | 0.00 | 7.33 | 0.00 |
| 1st 3 days   | 10.00 | 1.67 | 84.48 | –– | –– | –– | –– |
| 7 days       | 9.33 | 1.33 | 87.01 | –– | –– | –– | –– |
| Mean         | 1.83 | 1.83 | 85.75 | –– | –– | –– | –– |
| 2nd 3 days   | 10.33 | 1.00 | 90.91 | –– | –– | –– | –– |
| 7 days       | 10.67 | 0.33 | 96.75 | –– | –– | –– | –– |
| Mean         | 10.50 | 0.67 | 93.83 | –– | –– | –– | –– |
| 3rd 3 days   | 11.67 | 0.67 | 93.75 | –– | –– | –– | –– |
| 7 days       | 13.67 | 0.33 | 96.75 | –– | –– | –– | –– |
| Mean         | 12.70 | 0.50 | 95.30 | –– | –– | –– | –– |
| General average | 8.33 | 1.00 | 91.61 | –– | –– | –– | –– |
| 4th 3 days   | 13.67 | –– | 0.00 | 100.0 | –– | –– | –– |
| 7 days       | 14.67 | –– | 0.00 | 100.0 | –– | –– | –– |
| Mean         | 14.17 | –– | 0.00 | 100.0 | –– | –– | –– |
| 5th 3 days   | 13.31 | –– | 0.33 | 97.73 | –– | –– | –– |
| 7 days       | 14.67 | –– | 0.00 | 100.0 | –– | –– | –– |
| Mean         | 13.99 | –– | 0.17 | 98.87 | –– | –– | –– |
| 6th 3 days   | 13.67 | –– | 0.00 | 100.0 | –– | –– | –– |
| 7 days       | 14.00 | –– | 0.67 | 98.56 | –– | –– | –– |
| Mean         | 13.84 | –– | 0.34 | 99.28 | –– | –– | –– |
| General average | 14.00 | –– | –– | –– | 99.38 | –– | –– |
| 7th 3 days   | 13.67 | –– | 0.33 | 97.78 | –– | –– | –– |
| 7 days       | 18.00 | –– | –– | –– | 0.67 | 96.63 | –– | –– |
| Mean         | 15.84 | –– | –– | –– | 0.50 | 97.21 | –– | –– |
| 8th 3 days   | 21.33 | –– | –– | –– | 1.00 | 95.74 | –– | –– |
| 7 days       | 19.33 | –– | –– | –– | 1.33 | 93.73 | –– | –– |
| Mean         | 20.33 | –– | –– | –– | 1.17 | 94.74 | –– | –– |
| 9th 3 days   | 18.61 | –– | –– | –– | 0.67 | 96.75 | –– | –– |
| 7 days       | 20.67 | –– | –– | –– | 0.33 | 98.04 | –– | –– |
| Mean         | 19.67 | –– | –– | –– | 0.50 | 97.40 | –– | –– |
| General average | 18.61 | –– | –– | –– | 0.72 | 96.45 | –– | –– |
| General reduction | 95.81 | –– | –– | –– | –– | –– | –– | –– |

*R%, reduction percentages during 3 successive weeks

Rotations are a common tactic for usage in managing pesticide resistance and considered to have a good recorded in slowing down the evolution of resistance. It is also necessary to rotate the use of active substances with various modes of action (chemical group) in the IPM tactic. So, the insecticide should be selected carefully, especially in the early growth stages of the crop (IRAC 2017, 2019; Bassi et al. 2016).

The utilization of pheromone traps in monitoring *T. absoluta* adult, it might be possible to determine...
the ideal time for spraying pesticides, which leads to the reduction and rational use of insecticides. In addition, mass trapping techniques can be used with appropriately (Braham 2014). Pheromone traps are not only used for detection of the insect but also for controlling the T. absoluta population through mating disruption and mass annihilation (Ghoneim 2014; Retta and Berhe 2015).

Our findings are in agreement with El-Aassar et al. (2015) who found that using sex pheromone combined with other insecticides in controlling the leafminers infestation on tomato plants showed promising results, since sex pheromone traps were very effective in monitoring as well as mass trapping this pest, and it was useful in determining the most suitable time to begin spray insecticide.

As a rule, in chemical application, it is prudent to rotate different active ingredients and not to mix them at once to avoid the build up of resistance against the pesticides. The suggested active ingredients for rotation in the management of T. absoluta include using Radiant (Spinetoram) which exhibited an effective control of lepidopteran insects after a short time of treatment. It has a novel mode of action (neural mechanism), disrupting acetylcholine neurotransmission, and it has effects on a γ-aminobutyric acid (GABA) neurotransmitter agonist. It kills insects by hyper-excitation (causing tremors, paralysis) of the insect nervous system (Braham et al. 2012; Babar et al. 2016).

After that Coragen (Chlorantraniliprole) which works as both contact and stomach effects, it also has systemic translocation in the plant (phloem/xylem mobility) after soil application and has transaminar activity and penetrates the cuticle/epidermis. It induces muscular paralysis through disturbing the calcium balance, block sodium channel in nerve axon, and inhibit propagation of nerve potential; this explains its effect on the reduction of T. absoluta infestation.

The results of Valchev et al. (2013), Silva et al. (2016), Shiberu and Getu (2017), and Roditakis et al. (2018) are closed with our results where they determined a very good biological activity of Coragen towards the larvae of T. absoluta after 48 h of application.

The superiority of Emperor (emamectin benzoate), which has a high potency against a broad spectrum of lepidopterous pests, may be due to its predominantly to potentiating and/or direct opening of glutamate-gated chloride channels and the high affinity with the target site, and it may have irreversible effect, as well as, it binds to multiple sites (including glutamate and GABA) in insect chloride channels. Consequently, the insect is paralyzed irreversibly and stop feeding within hours of ingesting reaching to 2–4 days, so it prevents crop damage (Gacemi and Guenaoui 2012; Hamdy and El-Sayed 2013; Berxolli and Shahini 2018).

The utilizations of natural compounds such as plant essential oils are considered as an alternative to chemical pesticides because of their lower toxicity to the non-target organisms and low persistence in the environment (Chegini and Abbasipour 2017).

These natural chemicals (essential oils) have multiple modes of action, including antifeedant and repellent activities, antimoulting and respiration inhibition, growth and fecundity reduction, and cuticle disruption. So, they can act as a contact, fumigant,
repellent, antifeedant, and oviposition inhibitory toxicants (Sarwar and Salman 2015).

Rosemary essential oil is primarily composed of a mixture of monoterpenes such as 1.8-cineol (37.6 %) which represents the major compound followed by camphor (20.2%) and then α-pinene (15.7) that known to have insecticidal activities. Accordingly, the most interesting results of our study on the high toxicity of rosemary oil to T. absoluta can be readily attributed to more than one or two active principles (Yang et al. 2014).

Comparing the mode of action of the most effective naturally based formulated compound Rosa with the most efficient non-traditional chemical insecticides Emperor (emamectin benzoate), it is obvious that they have the same mode of action. They potentiate the actions of GABA at a recombinant insect ion tropic GABA receptor, which perhaps the most sensitive target site in T. absoluta that can affect by these compounds. So, we can conclude that either emamectin benzoate or Rosa have the same mode of action, so, this naturally based formulated compound has a good performance.

The superior benefits of this tactic depend on the direct production returns, the treatments with different insecticides resulted in a significantly maximum yield as well as the highest cost benefit ratio when compared with the untreated control one. Also, the maximum marketable yield and maximum additional income over control were obtained from the control treatments, and it gives the highest net profit over control as well as the maximum increase of the cost benefit ratio. These results are in conformity with Patra et al. (2009), Deshpande et al. (2010), Shivanna et al. (2011), and Dayaram (2014).

Conclusions
In this respect, according to the number of insects caught, it is clear that uses of insecticides or botanical formulation affect the insect infestation and consequently affect the number of captured insects/trap. To overcome or mitigate the incidence of insecticide resistance, it is necessitating rotating between several insecticides which have various modes of action that kill pests through different ways, especially when using several sprays during the season.

Integrated pest management (IPM) is the most useful method using rotating between chemical control in combination with other tactics such as mass trapping with sex pheromones and natural insecticides. The usage of IPM tactics in controlling the pests such as T. absoluta can increase the yield production and the quality of the marketable fruits.

So, during this study, the succeed IPM tactics in the management of T. absoluta were conducted, such as sex pheromone and sticky traps for monitoring and mass trapping the insects, associated with selective non-conventional insecticides (during vegetative stage) and formulated essential oils (during fruiting stage) as well as estimate the tomato yield production.

Abbreviations
IPM: Integrated pest management; EC: Emulsifiable concentrate; SC: Suspension concentrate; EW: Water based emulsion; Fed.: Feddan; LC: Lethal concentration; IRAC: Insecticide Resistance Action Committee; UN: Unknown

Authors’ contributions
M. A. Kandil, E. A. Sammour, N. F. Abdel-Atiz, E. Agarny, A. M. El-Bakry, and N. M. Abdelmaksoud designed experiments, carried out the biological experiments, analyzed the data, and wrote the article. All authors read and approved the final manuscript.

Funding
This research was financed by the National Research Centre, Egypt, Project No. 11030140.

Availability of data and materials
All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate
Not applicable (this study does not involve human participants, data, and tissue).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 11 December 2019 Accepted: 2 July 2020

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