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Effect of destruxin on the population reduction of green peach aphid *Myzus persicae* (Hemiptera: Aphididae) and the predator *Coccinella undecimpunctata* (Coleoptera: Coccinellidae) in tomato fields

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Abstract

Background: Destruxin is the toxin of some entomopathogenic fungi.

Objective: The efficiency of two destruxins, Destruxin A-*760* and Destruxin A-*724*, was evaluated against the green peach aphids *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) pests in tomato plants in two different climatic Governorates El-Sharkia and El Behira. Also, their safety levels to the predator *Coccinella undecimpunctata* L (Coleoptera: Coccinellidae) were studied under laboratory and field conditions.

Materials and methods: Two destruxin were applied on the target pests and its predators under laboratory and field conditions. Six aqueous concentrations of the two destruxin were prepared: 2.000–0.125 ppm. One day, adults of *C. undecimpunctata* and second instar nymphs of *M. persicae* were used for evaluation of the pathogenicity and efficacy of Destruxin A-*760* and Destruxin A-*724*. This was accomplished by different techniques, a spray technique to evaluate contact effect and a feeding technique to evaluate oral toxicity.

Results: Results showed that, under laboratory conditions, LC_{50} values for Destruxin A-*760* and Destruxin A-*724* were 58 and 66 ppm, respectively against *M. persicae*. Under field conditions, the percentages of infested plants with *M. persicae* were significantly decreased after treatments with both Destruxin A-*760* and Destruxin A-*724* as compared with the corresponding controls. In Nobaryia, weights of tomato yield were 3158 and 3988 kg/feddan (F) when Destruxin A-*760* and Destruxin A-*724* whereas the control yielded 2169 kg/F in the corresponding controls, respectively. While the corresponding yield in EL Sharkia (Zagazig) were 3569 and 3599 kg/F following the same order as compared to 2169 ± 36.82 and 2000 ± 80.54 kg/ F, respectively in the control. The study showed that *C. undecimpunctata* exhibits relatively high and reasonable resistance to Destruxin A-*760* and Destruxin A-*724* at their highest lethal concentration (LC) (i.e., 44 ppm) for treated insects.

Keywords: Destruxin A-*760*, Destruxin A-*724*, *Myzus persicae*, *Coccinella undecimpunctata*, Egypt, Tomato pests, Entomopathogenic fungi toxin

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Introduction

The tomato crop (*Lycopersicon esculentum*) is an important vegetable crop in Egypt; it belongs to Solanaceous. This plant is usually infested in Egypt with many destructive pests, especially the most destructive one which is the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) Blackman and Eastop (2006). These pests transmit several viruses to many economic crops (Namba and Sylvester 1981; Berry 1998). This species causes great damages and diseases to the leaves and fruits (Filotos et al. 2004). *M. persicae* is a small green aphid causing a decrease in growth, shriveling of the leaves, and the death of various tissues (Namba and Sylvester 1981; Berry 1998). The green peach aphid is found worldwide, although it is less tolerant of colder climates and over winters through its eggs, which are laid in trees of the genus *Prunus* (Howe and Jander 2008). Destruxin is the toxin of the fungi (*Metarhizium anisopliae*). This toxin causes death to many serious pests, which exhibit a variety of insecticidal actions (Roberts 1981).

The toxin called destruxin is a cyclic hexadepsipeptide produced by the fungus *Metarhizium anisopliae*. Destruxin (DEX) causes paralysis and a speedy death to the harmful pests and also it causes suppression of the insect immune system (Odier et al. 1992). Also, destruxin causes an inhibitory activity on the leukemic cell production, decreasing the number of cells in G2/M phase (Odier et al. 1992). Destruxins (DEX) cyclic hexadepsipeptide mycotoxin which have insecticidal and phytotoxic activity. Sabbour and Shaurub (2018) found that the destruxin treatments and nano destruxin decrease the infestations with *S. littoralis* under laboratory conditions. Under field conditions, the nano destruxin significantly decreased the infestations number of *S. littoralis* in the cotton field. Thungrabeab and Tongma (2007) reviewed the research of several authors dealing with the differential susceptibilities of many natural enemies to various fungal species. They concluded that some genera or species of fungi could be specific and might inflict only certain types of hosts. They also mentioned that the fungus *Beauveria bassiana* was not pathogenic to *Coccinella undecimpunctata* and *Chrysoperla carnea*.

The present work aims to evaluate the efficacy of destruxin against *M. persicae* and their main efficient predator (*C. undecimpunctata*) in tomato fields in Egypt.

Materials and Methods

Insect cultures

Pests

M. persicae were reared on small potted tomato plants inside cylindrical glass cages (15-cm diameter × 40-cm height), covered with muslin, under controlled conditions (26 ± 2 °C and 65 ± 5% RH).

Predator

The stock culture of the *C. undecimpunctata* started with adults that` were collected from aphid-infested tomato cultivars in Nobaryia, Egypt. Each of the 5 adults was kept in 2-L glass jars. The jars were supplied with fresh lettuce leaves infested with aphids for feeding. The jars were covered with muslin cloth held in position by rubber bands. Food was renewed every other day. The jars were checked daily for eggs. The eggs were collected and transferred to Petri dishes (20-cm diameter) till hatching. Neonate larvae were transferred individually to plastic cups with ample amount of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs till reaching the proposed experimental stage (second larval instar). Unused larvae were left in 2-L glass jars (5 each) with supply with small duranta branches carrying different stages of aphids for feeding till maturation.

Destruxin

Destruxin A-*760* and Destruxin A-*724* obtained from Shanghai Fuang Agrochemical Co. Ltd (99.9% purity) and prepared according to Guan et al. (2008). Six aqueous concentrations of both destruxins were prepared: 2.000, 1.000, 0.750, 0.500, 0.250, and 0.125 ppm. Fresh tomato leaves were dipped in each concentration for 10 s and left to dry at room temperature. Treated leaves were offered to third nymphal instars (20 nymphs/concentration). A parallel control of non-treated tomato leaves dipped in distilled water was run. Each treatment was replicated five times. The percentage of mortality was recorded after 7 days (Sabbour and Shaurub 2018) of treatment and corrected against that of the control according to the method described by Abbott's formula (Abbott 1925). Corrected mortality was subjected to probit analysis (Finney 1971) to determine the LC50 value. All experiments were run under the aforementioned laboratory conditions.

Treatment of pests

Fresh tomato leaves were sprayed with the desired Destruxin *A-760* and Destruxin *A-724* concentration (3 shots as spurts/leaf) (Matter et al. 1993), left to dry and placed in 1-L plastic containers (one each). Then, 20 nymphs of *M. persicae* were placed on each leaf. Five containers (replicates) were used/concentration/microbial pathogen (destruxin)/aphid. Each container was covered with muslin and incubated at 25 °C, thereafter, untreated leaves were introduced in the plastic containers to allow the gentle transfer of survivors to them and the previously treated leaves were discarded. Untreated leaves were placed in plastic containers sprayed with water only and used as the control treatment. The experiment was replicated four times. The percentages of mortality were calculated after 7 days and corrected and treated as above.

Treatments of predator

One-day-old adults, second instar larvae of *C. undecimpunctata*, and second instar nymphs of *M. persicae* were used for evaluation of the pathogenicity and efficacy of Destruxin A-*760* and Destruxin A-*724*. This was accomplished by different techniques.

A—Spray technique to evaluate the contact effect

B—Feeding technique to evaluate oral toxicity, either obligatory (no choice) (exposure to treated preys only) or free choice exposure to both infected and uninfected preys, and to investigate whether the predator has the ability to distinguish between the infected, uninfected preys, and not

Spray technique

Groups (20 predators of 1-day-old adults of *C. undecimpunctata* or 1-day-old second nymphal stage/group) were placed in a Petri dish (19-cm diameter) and sprayed with the fungus at 2.00 ppm concentration level using a small atomizer, by three shots as spurts (Matter et al. 1993). The shots were directed to the insects at a 15-cm distance, then the insects were individually transferred gently, using tweezers, to plastic cups (5-cm diameter and 12 cm) with small water moist filter paper and aphid-infested tomato leaf. The cups were covered with muslin and incubated at 25 ° C. The filter paper and branches carrying aphids were renewed every other day. Five groups (20 individuals/group) from each stage were used for each destruxin.

The cups of each group were checked daily for insects showing signs of fungus infection. The death toll was recorded for two weeks post-treatment and mortality percentages were calculated in each case.

Obligatory and free-choice feeding techniques

Groups of 20 individuals of either adults of *C. undecimpunctata* or second instar nymphs of *M. persicae* per group were exposed either obligatory to contaminated diet (pathogen-treated aphids) or selectively to pathogen-treated and untreated aphids for 24 h. In case of free-choice feeding, 5 groups were used/pathogen/predator stage. The predator was kept starved for 4 and 6 h for *M. persicae*, respectively, then, each group was introduced in the middle of a 5-L glass jar with 2 branches of tafla carrying ample amounts of the pest (one branch was previously sprayed with the destruxins while the other branch was sprayed with water only). The two branches were placed on both sides of the glass jar facing each other to allow the predator individuals free-choice to feed on either treated or untreated aphids. Five glass jars (replicates) were used for each pathogen.

Regarding obligatory feeding, the same number of predators in each of 5 glass jars were used as mentioned above but offered only treated aphids. In both trials, the exposure period was 24 h. Then predators of every treatment and the control as well were transferred individually to plastic cups, offered untreated aphids, and checked daily for 14 days

Field experiments

Pests

Experiments were carried out to study the efficacy of the tested two destruxins against the target insect pests in two different areas that differ in climatic and soil factors: in Ibn Malek (El-Nobaryia region) with dry weather and sandy soil and in El-SharKia (Zagazig) with wet weather and clay soil. Tomato (Var. Bio-Bride) was planted at the first of April in an area of about 1200 m^2, divided into 12 plots of 100 m^2each. Four plots were assigned for each pathogen, while 4 plots were treated with water and used as controls Destruxin A-*760* and Destruxin A-*724* were applied at 5 ppm concentration and 5 L/plot. Treatments were performed in a randomized plot design at the sunset with a 5-L sprayer. Three applications were made at 1-week intervals at the commencement of the experiment, then 20 samples of plants were randomly collected every week from each plot and transferred to the laboratory for examination. The average number of each of the tested pests/sample/plot/treatment was calculated 20, 50, 90, and 120 days post first application. The infestation of aphids was then determined in each case.

After harvest, the yield of each treatment was weighed as kg/F. Yield loss was calculated according to the following equation:

$$\text{Yield loss} = \frac{\text{potential yield–actual yield}}{\text{Potential yield}} \times 100$$

Potential yield was that of which gave the best results among the tested pathogens Destruxin A-760 and was taken as a base for comparison with the other treatments.

Predator

Seedlings of tomato plants were sown in rows (ca 50 cm apart) in ca 0.5 F located in the El Sharkia Governorate. One-month-old plants were found highly infested with *M. persicae*. The cultivated area was divided longitudinally into 3 areas (ca Kirat/each), separated from each other by uncultivated bare and had 4 m/width. One area was used for each entomopathogen toxin tested and the check as well. Each pathogen was sprayed at the rate of 5 ppm/F, using a high pressure hand held gun. The concentration of the Destruxin *A-760* and Destruxin A-*724* was about 5 ppm. (This concentration was previously achieved more than 80% mortality in both pests in laboratory experiments.) Three applications were made first at 1-week intervals. Then, count of *C. undecimpunctata* on nymphs and adults of *M. presicae* were carefully counted on site in all tomato plots visually, hand picking, and finally,

Table 1 Effect of the destruxin tested against *M. persicae* under laboratory conditions

Tested insect	Tested destruxin	LC_{50} ppm	95% confidence limits
M. persicae	Destruxin A-760	58	22–88
	Destruxin A-724	66	33–99

sweeping net (25-cm diameter). The counts were made just before the first application and 1, 2, and 3 weeks post last application. The predators were placed again after each count on their previous location in the corresponding plant site. Fifty tomato shrubs (10 from each of 5 raws) per each treated area and the control as well were arbitrarily chosen/each time interval. The average number of predators/50 plants/time interval was calculated in each case. The increase or decrease in the population of the predator/50 plants as compared with the check was calculated according to Hendrson and Tilton's (1955) equation as follows:

$$\%\text{increase or decrease in population density} = \frac{Ca}{Cb} \times \frac{Tb}{Ta} - 1 \times 100$$

where Ca = population density in the treated area before treatment, Cb = population density in the treated area after treatment, Ta = population density in treated before treatment, and Tb = population density in the treated area after treatment.

Results

Table 1 shows that the LC50 of *M. persicae* was 58 and 66 ppm when treated with Destruxin A-760 and Destruxin A-724, respectively.

Under field conditions in both localities, *M. persicae* infection was significantly decreased. After 20 days of applications, the infections with the target pest obtained, 14 ± 4.7 and 11 ± 7.9 individuals in Destruxin A-724. Treated plots in Ibn Malek Nobaryia (Behira) and EL Sharkia (Zagazig), respectively. The corresponding data obtained 0 and 6 ± 1.2 individuals after Destruxin A-760. Treatment plots as compared to 56 ± 6.6 and 65 ± 8.9 individuals in the corresponding treatment areas.

In plots treated with Destruxin A-760, the infestations with *M. persicae* significantly decreased to 23 ± 4.5 and 36 ± 7 individuals, respectively, as compared to 152 ± 7.8 and 163 ± 4.7 individuals in the control, after 120 days of applications in both two governorates. The same obtained in case of Destruxin A-724 application after 120 days, the number of target insects were significantly decreased to 36 ± 7.7 and 46 ± 6.6 in Ibn Malek Nobaryia and EL Sharkia as compared to 152 ± 7.8 and 163 ± 4.7 individuals following the same order (Table 2).

Effects of Destruxin A-760 and Destruxin A-724 on the predator on *C. undecimpunctata* were shown in Table 3, all of the experiment results cleared that the predator *C. undecimpunctata was* not affected to both destruxins treatments. This predator *C. undecimpunctata* proved a higher resistance against destruxin treatments Table 3. Our results show that the predator preys on nymphs which are more susceptible to that on the adult stages of

Table 2 The infestation of tomato plants with *M. persicae* after treatment with Destruxin A-724 and Destruxin A-760 under field conditions

Treatments	Days after first application	Means number of *M. persicae* in application areas during 2018	
		Ibn Malek Nobaryia (Behira)	EL Sharkia (Zagazik)
Control	20	56 ± 6.6	65 ± 8.9
	50	76 ± 7.7	189 ± 7.7
	90	94 ± 7.7	198 ± 7.6
	120	152 ± 7.8	163 ± 4.7
Destruxin A-*724*	20	14 ± 4.7	11 ± 7.9
	50	19 ± 2.8	25 ± 8.3
	90	34 ± 4.2	35 ± 7.8
	120	42 ± 7.1	46 ± 6.6
Destruxin A-*760*	20	0	6 ± 1.2
	50	7 ± 2.6	10 ± 3.7
	90	16 ± 5.3	20 ± 9.4
	120	23 ± 4.5	36 ± 7.7
F test	29.7		24.7
Lsd 5%	12.7		19.7

Table 3 Determination of the effect of (Destruxin A-*760* and Destruxin A-*724*) on developmental stages of *C. undecimpunctata* after feeding *M. persicae* nymphs and adults

Pathogen	Mortality of infected *M. persicae* M ± SE					
	Nymphs			Adults		
	Treatment					
	Spray	Ingestion of the treated food		Spray	Ingestion of the treated food	
		Obligatory (no-choice)	Selection (choice)		Obligatory (no-choice)	Selection (choice)
Destruxin A-760	79.10 ± 7.61	20.6 ± 3.49	11.8 ± 1.76	25.6 ± 3.19	17.3 ± 2.71	7.5 ± 6.51
Destruxin A-724	21.2 ± 4.45	31.2 ± 4.45	21.4 ± 2.90	37.6 ± 7.71	30.2 ± 4.49	20.8 ± 2.82
F-test	20.12					
LSD 5%	12.23					

C. undecimpunctata. Under laboratory conditions, the nymph's mortality of *M. persicae* obtained 79.10 ± 7.61 and 21.2 ± 4.45 after treated with Destruxin A-760 and Destruxin A-724, respectively (Table 3). In case of adult stages, they significantly decreased by 0.6 times treated with the following in the same order.

In general, the indirect treatments by feeding by either of the treated of the prey *M. persicae* only (required) or by free-choice feeding on either treated or untreated prey (selectivity) revealed that the required ingestion of Destruxin A-760-infected prey of *M. persicae* caused a mortality percentage of 2.56 and 2.31 times that obtained from those of the given free-choice ingestion (selection treatment) for adult and nymph of *C. undecimpunctata* predators, respectively. The corresponding ratios for Destruxin A-724 were about 1.35 and 1.86, respectively. This indicated that the predator, particularly the adult predator, has a greater ability to recognize between the Destruxin A-760-treated prey and non-treated ones than Destruxin A-724fungus, which indicates that the adult predator can avoid Destruxin A-760 which infected the prey much more than that of the adult predator *C. undecimpunctata* avoiding the Destruxin A-724-infected prey. It is worth mentioning that no death from both destruxin's infection was encountered in the check within the experimental period.

Under field conditions the experiments results showed that the population density of the predator *C. undecimpunctata* in the Destruxin A-760-treated area showed 36.22 and 19.81% reductions, 1 and 2 weeks after the last application, respectively. While the corresponding densities in the control area showed a 21.33 and 2.7% increase. However, the population, 3 weeks after the last application surpassed that of the check, showing a 6.36% increase (Table 4). In the *I. fumosorosea*-treated area, severe reductions in the population densities (63.33 and 43.99%) were estimated in the first and second weeks after the last application, respectively. There was less reduction (21.42%) estimated 3 weeks after the last application. However, the relatively higher reductions in predator densities in the treated areas, in the first weeks after the application. percentages increased or decreased in the *C. undecimpunctata* population as compared with the check according to Hendrson and Tilton (1955).

Field application of both the two bio-insecticides showed that in the control plots, the estimated yield weights were 2169 ± 36.82 and 2000 ± 80.54 kg/F in Ebn-Malek Nobaryia (Behira) and EL Sharkia governorate (Zagazig) during the season of 2018, respectively. While in Destruxin A-760- and Destruxin A-724-treated plots, the estimated weights of the tomato yields were 3988 ± 34.31 and 3158 ± 42.57 kg/F, respectively, in the Ebn-Malek El-Nobaryia (Behira) region. In the El Sharkia (Zagazig), the untreated plots recorded 1990 ± 80.54 kg/F but the weight showed a significant increase post of the Destruxin A-760 and Destruxin A-724 treatments.

Table 4 The effect of destruxins on *C. undecimpunctata* (all stages)/50 tomato shrubs after successive post toxin application in tomatoes

Post application (Weeks)	Treatments Average number of *C. undecimpunctata* ± SE				
	Control	Destruxin A-760	Destruxin A-724	% increase (+) or decrease (–)*	
Just before first Application	18.75 ± 3.36	21.00 ± 2.55	20.00 ± 1,58	Destruxin A-760	Destruxin A-724
One week after last application	22.75 ± 1.70	16.25 ± 2.25	7.5 ± 1.71	– 36.22	– 69.09
Two weeks after last application	19.25 ± 1.54	17.29 ± 1.66	11.5 ± 0.96	– 19.81	– 43.99
Three weeks after last application	17.00 ± 2.12	20.25 ± 1.65	14.25 ± 1.93	+ 6.26	– 21.42

*Percentages of increase or decrease in *C. undecimpunctata* population density as compared with the check according to Hendrson and Tilton (1955)

Table 5 Weight of harvested tomatoes and percentage of yield loss during season 2018 post the two-destruxin treatments of *M. persicae* in two governorates

Treatments	Ebn-Malek Nobaryia (Behira) Tomato weight		EL Sharkia (Zagazig) Tomato weight	
	Weight tomatoes (Kg/feddan)	% yield loss	Weight tomatoes (Kg/feddan)	% yield loss
Control	2169 ± 36.82	45	2000 ± 80.54	44
Destruxin A-760.	3988 ± 34.31	–	3599 ± 65.32	–
Destruxin A-724.	3158 ± 42.57	20	3569 ± 69.33	0.8
F values	31.42		32.40	
LSD 5%	81		80	

The percentages of yield loss in the untreated plots were 30 and 33% in the Ebn-Malek El-Nobaryia (Behira) and the El Sharkia (Zagazig), respectively (Table 5).

Discussions

The present study showed that *C. undecimpunctata* exhibits relatively high and reasonable resistance to the tested entomopathogenic fungi *Destruxin A-724* and *Destruxin A-760* infections, respectively, even when exposed to a lethal concentration for the prey insects.

Thungrabeab and Tongma (2007) concluded that some genera of fungi could be specific and might inflict only on certain types of hosts. They reported the work of James and Lighthart (1994) who declared that the fungus *N. rileyi* exhibits host preferential infection in lepidopterous larvae. Also, they found that *M. anisopliae*, *B. bassiana*, and *P. fumosorosea* fungi have potential to infect *Hippodomia converges* (*coccinellidae*) whereas *N. rileyi* did not. Goettel et al. (1990) found that some commercial formulation of the entomopathogenic fungi can control aphids and thrips with low impact on non-target insects. Todorova et al. (1994) found that different strains of (*B. bassiana*) fungus showed different effects on the two Coleopterous predatory insects due to the host response of the insects. Sabbour and Sahab (2007) control *Agrotis ipsilon* and *Heliothis armigera* by the entomopathogenic fungi. Sabbour and Abdel-Rahman (2007) found that the two microbial control agents reduce the number of sugar beet pests under laboratory and field conditions. Sabbour (2007a, 2007b) found that the entomopathogenic fungi *Nomuraea rileyi* and *Isaria fumosorosea* proved highly pathogenic to aphids and the natural enemies *Coccinella* spp. were not affected by the fungi treatments. Poprawiski et al. (1998) found that *Serangium parcestosrum* (Coccinellidae) had lower survival potential when sprayed with Zagazig (*B. bassiana*) fungus than that with *P. fumosorosea* fungus. Shanthakumar et al. (2010) considered that in spite of the great virulence of *N. rileyi* against *Spodoptera litura*, the pathogen proved reasonable safety to *Trichogramma chilonis*. It did not cause a reduction in their parasitization percentages.

The present results also indicated that the predator, *C. undecimpunctata*, particularly adult predators can distinguish between fungus infected from non-infected preys and they almost avoid treated ones, especially if given free choice feeding. This, however, was more pronounced in the case of *N. rileyi* than *P. fumosorosea*. Such observed phenomenon in our investigations was viewed by many authors. It was mentioned that predators, when given free choice to feed upon fungus-treated or untreated aphids, predation on infected preys was less than uninfected ones (Baverstock et al. 2007). Also, Roy et al. (2010) and Goettel et al. (1990) proved that *C. septempunctata* adults avoid contacting with leaf and soil surface inoculated with *B. bassiana* fungus and mycosed cadavers. The predator was positioned away from mycosed cadaver than uninfected ones.

Nevertheless, some researches indicated several adverse effects of some entomopathogenic fungi against some natural enemies. It was considered that *C. septempunctata* was somewhat susceptible to *B. bassiana* (Haseeb and Murad 1997; Delete et al. 1995). Farag (2008) considers that some entomopathogenic formulations of *B. bassiana* have deleterious effects on *C. undecimpunctata* if applied at high-concentration levels. However, different views about the safety of entomopathogenic fungi, declared by many authors, might be due to the relative efficacy of the fungus or its isolates on pests the exhibit different susceptibilities, bionomics, and characters as well as types of assessment and application rates.

Conclusion

Destruxin A-*760* and Destruxin A-*724* are a promising control against *M. persicae* as they reduce the pest population under laboratory conditions. On the other hand, in the population under field conditions, the pathogen significantly reduced the target pests. Pathogen Destruxin A-760 and Destruxin A-724 tested non-significant effects on the predator's populations.

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Author's contribution
The author equally contributed in all the article parts. The author read and approved the final manuscript.

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