# Probing the Effects Destruxins on Leopard *Zeuzera Pyrina* (L.) (Lepidoptera: Cossidae), in Olive Trees

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## Abstract:
Olive tree is subjected to attack by many insect pest species that effect on the yield quality and quantity. Among the most common pest species surveyed in Egypt is; the leopard Zeuzera pyrina (L.) (Lepidoptera: Cossidae), which considered a serious pest in olive fields causing a lot of damage and loss in olive trees. Destruxin consist of an a-hydroxyl acid and five amino acid residues. It is a cyclic hexadepsipeptide produced by fungus causing paralysis and death in insects. It causes the infestations decrease of many insect pests. The effect of destruxins was tested under laboratory and field conditions against Z. pyrina. Results showed that the LC50 of Destruxin recoded 139 ppm when the 3rd larval instars Z. pyrina treated with different concentrations. When the Isolate Destruxin A-304 applied on the target pests the LC50 recorded 33 ppm. Under field conditions, the infestations were significantly decreased to 7.0 $\pm$3.2 and 7.0 $\pm$4.2 larvae after treated with Isolate Destruxin A-304 in Ebn Malek and Ismailia, respectively. In the same last places the Isolate Destruxin A-366 application showed a significant decrease in the pests infestations reached to 45.0 $\pm$ 1.2 and 41.0 $\pm$ 1.2 larvae as compared to 99.0 $\pm$ 1.2 and 99.0 $\pm$ 1.2larvae in the control. The yields weights in both two regions were significantly increased as the result of Isolate Destruxin A-304 applications.

## Keywords:
Zeuzera Pyrina, Destruxin, Isolates, Olive

## 1. Introduction

Destruxin is a cyclic hexadepsipeptide produced by fungus causing paralysis and death in insects. They are the only mycotoxins detected in the insect body at advance stages of infection in sufficient quantities to cause death May suppress the insect immune response. Possesses an inhibitory activity on leukemic cell proliferation, and decreases the number of cells in $G_2$/M phase. [1, 2, 3] It is virustatic against arboviruses. Indeed, the destruxins also act as effective insecticides against *Spodoptera litura* (Fab). They mediate specific down-regulation of antimicrobial peptides by targeting the insect's innate immune signaling pathway. Remarkable changes in the status of several antioxidant enzymes (catalase, peroxidase, ascorbate oxidase, and superoxide dismutase).

Destruxin considerd a microbial insecticide with potent bioactivity against many insect pests. Olive has become one of the important economical crops in Egypt. Its cultivated area has been expanded largely in the last decade, particularly in new reclaimed arid areas (Western side of the Nile). Its area reached 49000 Hectares in 2010 (productivity = 6327 Kg/ Hectare) [4]. The leopard moth, *Zeuzera pyrina* (L.) (Lepidoptera: Cossidae), is a harmful pest for many fruit trees (e.g., apple [*Malus* spp.], pear [*Pyrus* spp.] peach [*Prunus* spp.], and olive [*Olea*]. Recently, it caused yield losses in the newly established olive orchards in Egypt, including the death of young trees [5]. Chemical control has shown limited efficiency against this pest. Imidacloprid is a systemic insecticide with translaminar activity with contact and stomach action. Readily taken up by the plant and further distributed acropetally, with good root-systemic action. Imidacloprid is used for controlling the sucking insects, including rice-, leaf- and planthoppers, aphids, thrips, Lepidopterous and whitefly. Also effective against soil insects, termites and some species of biting insects, such as rice water weevil and Colorado beetle. The mentioned compound has no effect on nematodes and spider mites. Imidacloprid is also used as a seed dressing, as soil treatment and.This work aims to control leopard *Zeuzera pyrina* (L.) larvae by using destruxins compound.

## 2. Materials and Methods

### *2.1 Rearing of Zeuzera pyrina (L.)*

The larvae ($1^{st}$, $2^{nd}$, $3^{rd}$) were collected from heavy infested trees during May, April, rearing technique according to [5]

### *2.2 Mycotoxin preparation*

Two isolates *Metarihizium anisopliae* Destruxin (A-304 and A-336,) were obtained in a series of soil screening experiments by using 300 samples [6]. The isolates were inoculated in 50 mL Potato Dextrose Broth (PDB) mediums for destruxin production. The medium was filtered using filter paper seven days after culturing. Then 10 mL chloroform was added and shakevigorously for 10 min. The supernatant evaporate and the residue was containing destruxin. The residues were dissolved in 10 mL distilled water and stored at -20 ℃ for further examinations

### *2.3 Destruxin*

Two types of Destruxin were tested 1.commercial one obtained Shanghai Fuang Agrochemical Co. Ltd. The $2^{nd}$ isolated from the fungus *Metarihizium anisopliae*, Isolate Destruxin A-304 and Isolate Destruxin A-304. Destruxin prepared into 6 concentrations 2, 1.5, 0.75, 1, 0.5, 0.25, 0.125 ppm. The target insect pest treated with the last concentrations. Dead insect pests were counted and removed from the cages daily for 21 days. Each treatment was replicated five times The percentages of mortality were calculated after seven days and corrected according to Abbott's formula [7], while the LC50 value was calculated through Probit analysis according to Finney equation [8]. All experiments were applied at (25±2oC and 65±5% R under laboratory conditions.

### 2.4 Field applications

The study was conducted from 2015 to 2016 in a densely planted olive orchard (240 ha, 336 trees/ha) located in two regions, El Nobaryia (Ebn Malek ) and Ismailia (Kassaseen).Each farm is divided into 88 isolated plots (3.0-3.5 ha, each) by windbreak hedges. Each plot is divided into 10 sectors 'strips', each 3 × ≈ 26 to 30 trees. Each strip combines three lines of one variety alternated by another strip 3 lines of the second variety and so on "strip cropping system". So the width of each strip is similar. The orchard has been established in 2015, it is drip irrigated and not in close proximity of apple plantation or any other known host plants of *Zeuzera* species.

Trees were approximately 3-4 m height, planted at 5 m distance along the row and 6 m distance between two lines. No chemical control was applied on monitoring or experimental plots during the experimental period. The Destruxin and isolated Destruxin -304 and isolated destruxin366 were applied at 200ppm. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at the sunset with a ten litre sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days from the application. Each treatment was replicated four times. Four plots were treated with water as the control. Random samples of leaves and fruits weekly collected from each treatment and transferred to laboratory for examination. The infestation of, *Z.pyrina* were estimated in each case.

After harvest, yield of each treatment was estimated as Kg/Feddan.

### 2.5 Statistical analysis

Data were statistically analyzed by *F*-test; LSD value was estimated, using SPSS tatistical program software.

## 3. Results and Discussion

Table 1 shows that Destruxin was about 4 times as toxic as Isolate Destruxin A-304 to *Zeuzera pyrina* third larval instars under laboratory conditions. Also data in table 1 clear that Destruxin recorde 1.5times toxic than Isolate Destruxin A-366. In comparison, the $LC_{50}$of Destruxin against the olive pests, *Z. pyrina* was 139, 33, 89 for Destruxin, Isolate Destruxin A-304 andIsolate Destruxin A-366 (Table1). In comparison, the $LC_{50}$ of Imidacloprid against the olive pests, *Ceratitis capitata* and *Pryas oleae*, was 221 and 200 mg/L, respectively [8].

***Table 1.*** *Effect of destruxin on Zeuzera pyrina (L.) larvae under laboratory conditions*

| Treatments | $LC_{50}$ | Slope | Variance | 95%confidence limits |
|---|---|---|---|---|
| Destruxin A-304 | 33 | 0.01 | 0.03 | 88-33 |
| Destruxin A-366 | 89 | 0.02 | 0.01 | 119-70 |

Field studies revealed that the rate of infestation of olive trees by *Z. pyrina* was significantly ($P < 0.05$) declined due to treatment with Destruxin Isolate Destruxin A-366 and Isolate Destruxin A-304 compared to control insects with the least infestation in case of treatment with nano-Imidacloprid at both Ibn Malek and Ismailia regions (Table 2). The least infestation was attained after 20 and 120 days of treatment with Imidacloprid and nano-Imidacloprid, respectively. Interestingly, the infestation decreased with the increase in time after treatment with nano-Imidacloprid. Similarly, [8] reported that Imidacloprid and nano-Imidacloprid reduced the rate of infestation by *C. capitata* and *P. oleae* in olive trees. Again, [9] recorded decreased infestation rate by potato tuber moth, *Phthorimaea operculella*, in plants treated with nano-fungi *Isaria fumosorosea* and *Metarhizium flavoviride*. Similar findings were also attained by [10] against *B. oleae*, *C. capitata* and *P. oleae* in olive trees treated with spinosad.

***Table 2.*** *Larval numbers of Z. pyrina after treatment with destruxin under field conditions throughout the two 2015 and 2016 seasons*

| No. of infested plants ± SE | | | |
|---|---|---|---|
| Treatment | Days after treatment | Ibn Malek | Ismailia |
| Control | 20 | 22.1 ± 5.7 | 22.1 ± 2.5 |
| | 50 | 71.1 ± 2.3 | 75.0 ± 2.2 |
| | 90 | 90.0 ± 4.4 | 95.0 ± 3.6 |
| | 120 | 99.0 ± 1.2 | 99.0 ± 1.2 |
| Isolate Destruxin A-304 | 20 | 10.0 ± 2.1 | 10.0 ± 2.5 |
| | 50 | 9.6 ± 2.3 | 9.3 ± 2.2 |
| | 90 | 9.0 ± 1.4 | 9.1 ± 3.6 |
| | 120 | 7.0 ± 3.2 | 7.0 ± 4.2 |
| Isolate Destruxin A-366 | 20 | 13.1 ± 5.1 | 13.1 ± 2.5 |
| | 50 | 25.1 ± 2.3 | 26.0 ± 2.2 |
| | 90 | 39.0 ± 4.4 | 40.0 ± 3.6 |
| | 120 | 45.0 ± 1.2 | 41.0 ± 1.2 |
| *F*-test | | 32 .1 | 30.4 |
| LSD (P = .05) | | 93 | 91 |

The weight of harvested olive fruits was significantly (P < 0.05) enhanced after treatment olive trees with destruxin Isolate Destruxin A-304 and Isolate Destruxin A-366 at Ibn Malek and Ismailia compared to control trees (Table 3). destruxin Isolate Destruxin A-304 and Isolate Destruxin A-366 increased the crop yield to, 4296.± 20.11, 5789.1 ± 70.10 and 4496.0 ± 21.11 Kg/ feddan respectively as compared to2121.0 ± 20.72 Kg/ feddanin the control at Ebn El Malek region. In Ismaila, the weight of olive after the corresponding treatments recored 45094.0 ± 31.18, 5994.0 ± 51.10 and 4999.0 ± 20.11 kg/ feddan, espectively as compared to 2004.0 ± 81.50kg/feddan in the control r (Table 3).

***Table 3.*** *Weights of harvested olive fruits after treatment with destruxin against Z. pyrina during 2015 and 2016 seasons at the experimented areas*

| Weight of yield (Kg/Feddan) | | |
|---|---|---|
| Treatment | Ibn Malek | Ismailia |
| Control | 2121.0 ± $20.72^{b}$ | 2004.0 ± $81.50^{b}$ |
| Isolate Destruxin A-304 | 5789.1 ± $70.10^{a}$ | 5994.0 ± $51.10^{a}$ |
| Isolate Destruxin A-366 | 4496.0 ± $21.11^{a}$ | 4999.0 ± $20.11^{a}$ |
| *F*-test | 40.1 | 37.4 |
| LSD (P = 0.05) | 87 | 87 |

These results are in consistence with those obtained by [8] for olive trees treated with Imidacloprid and nano-Imidacloprid and infested by *C. capitata* and *P. oleae*. Also, treatment of potato plants, infested by *P. operculella*, with nano-fungi *I. fumosorosea* and *M. flavoviride* increased the yield [9]. Similar results were obtained by [10] for spinosad-treated olive trees that were infested by *B. oleae*, *C. capitata* and *P. oleae*. [11], found that the olive weight increased after bioinscticid applications. [12], reported that nano-biopesticides application increase the productivity of the olive fruits under field conditions.

In conclusion, nano-formulation of Imidacloprid was more effective than Imidacloprid in controlling *B. oleae*. These results encourage the extension in the use of nanotechnology for insect pest control.

Figure 1, 2, show the infestation of the olive trees in the field by *Z. pyrina* which significantly decreased in both two places of experiments.

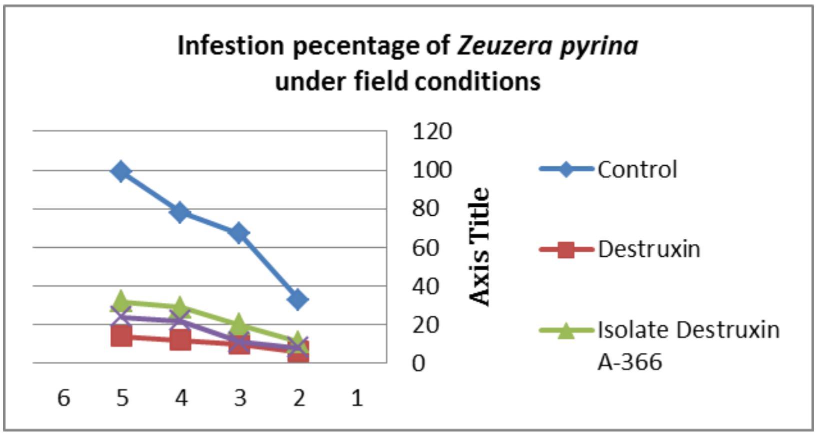


***Figure 1.*** *Infestion pecentage of Zeuzera pyrina under field conditions in Ibn Malek*

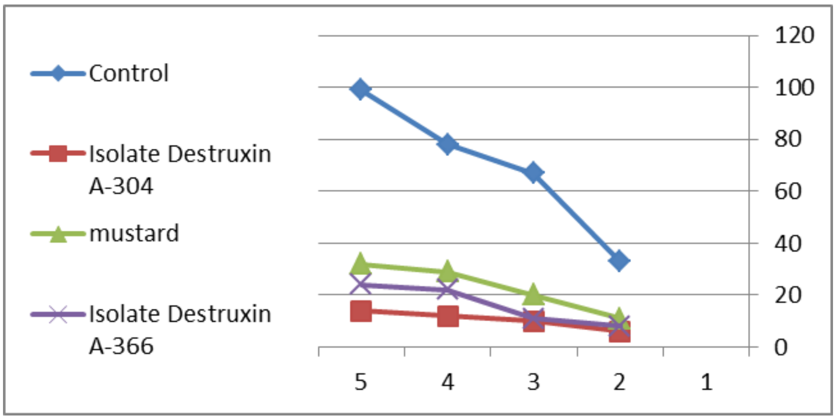


***Figure 2.*** *Infestion pecentage of Zeuzera pyrina under field conditions In Ismailia*

[11, 13] use the toxin of the fungus *Metarhizium anisopliae* against the olive insect pests and found that the toxin Destruxin could to control these pests under laboratory and field conditions. [14, 15, 16] control *P. oleae*, *B. oleae* and *Ceratitis capitat*a by Imidacloprid under laboratory and field conditions. They reported that the infestations of the three pests, reduced under field conditions. In [9, 10], reported that the three olive pests recoded a low application percentages in the field after *Nomuraea rileyi*, *Isaria fumosorosea*and Spinosad treatments under field and laboratory conditions. The same results agree with [17].

Although the mode of action of destruxins in insects is an unclear issue but altering the calcium cannels function has proposed in some investigations [18, 19]. Primarily, tetanic paralysis is the common symptom in insects causing by application of destruxin [19]. Opening the Ca2+ channels as a result of membrane depolarization by destruxin has been implicated as a cause of paralysis and death [20]. Humoral immune response seems to be specifically affected by destruxin [21]. Taken together these findings describe the probable reason for slow-acting this mycotoxin. Recording mortality data after the minimum of 72 h is a common procedure. Based on our data, three to four days after treatment appear to be appropriate final point for recording mortality. Expensive production of microbial biopesticides is one of the limiting factors for wide application of these agents [22, 23]. In many cases, entomopathogens are potential control agents against pests and use along with other component of integrated pest management [22, 24]. Therefore the dilutions of extracted destruxin are suitable candidates in these programs; they decrease application's cost of destruxin in an integrated procedure. 10-fold dilution of A-115 (Like other microbial agents, long period of lethal infection [22] is a disadvantage of destruxins. This make the LT50 value consider as a significance parameter. Citrus leaf miner produces four larval instars in shallow tunnels (mines) inside the leaves of young citrus trees [25]. The first three instars are active and feed within the mines, while the forth in star, no longer feed and produces silk from its mouthparts to form a pupal chamber. Growth of three stages takes about five to six days in summer [2,], but in different environmental conditions it may be longs one to three weeks [25]; Grafton-Cardwell, [26, 30, 31]. By considering the biology of CLM and the LT50s of isolates, destruxin have acceptable efficacy on CLM. Also, these measures suggest that three to four days after treatment is a suitable endpoint for Destruxin bioassay. [11, 27, 28, 29, 30], a found that the leopard larvae *Z. pyrina* decreased under laboratory and field conditions after nano- *Beauvericin* treatments. Sabbour 2017 b found the *Beauvericin* treatments increase the productivity of olive fruits under field conditions and decrese the infestions with olive pests. Sabbour and Shurab 2o17 use the imidclorprid aginst olive pest and recorded that, the yield of harvested olive fruits increased due to treatment with Imidacloprid and nano-Imidacloprid with the highest yield in case of trees treated with nano-Imidacloprid.

## 4. Conclusions

Three Destruxin were tested on *Z. Pyrina* under laboratory and field conditions. Conmmertial Destruxin, and two isolated destruxin and evaluated on *Z. pyrina*, results showed that the isolated two Destruxin is more effective on the target insect ests under laboratory and field conditions.

## Conflicts of Interest

There is no conflict of interest regarding the publication of this article.

## Acknowledgments

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