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Evaluating toxicity of nano-extracted Destruxin from *Metarhizium anisopliae* against the grasshopper *Hetiracris littoralis* in Egypt

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ABSTRACT

Under laboratory conditions, the LC_{50} of newly hatched nymphal stage recorded 200 $X10^4$ spores/ml and 129 X $X10^4$ spores/ml. For last nymphal state the LC_{50}s recoded 214X 10^4 and 258 X 10^4 spores/ml. Also, the LC_{50} of the adult female and male recorded, 233 and $257X10^4$ spores/ml and $279X10^4$ when treated with different concentrations of Destruxin and nano-destruxin, respectively. Also, under laboratory conditions, the LC_{50}s, were significantly decreased when the adult female of grasshopper *Hetiracris littoralis* treated with nao-destruxin and reached to 153X 10^4 spores/ml.

Under semi-field conditions, the percentage of infestations of *H. littoralis* significantly decreased to 1.0±0.3, 3±0.1, 5±3.0 and 10±2.9 individuals after treated with nano-destruxin in 20, 50, 90 and 120 days, respectively as compared to 15.2±2.9, 39±3.5, 66±9.6 and 98±6.6 individuals in the control.

Key words: Destruxin, *Metarhizium anisopliae*, microbial agent, grasshopper, *Hetiracris Littoralis.*

INTRODUCTION

The fungus, *Metarhizium anisopliae* (Metschnikoff) Sorokin produces some cyclic peptide toxins, destruxins, which exhibit a variety of insecticidal actions. Grasshopper *Hetiracris littoralis* is a major pest they feed on green plants and cause a lot of damages; therefore its effective and safe control is an important issue. Isolates of *M. anisopliae* were inoculated in Potato Dextrose Broth (PDB) mediums. Destruxin was extracted by adding chloroform. Grasshopper *Hetiracris littoralis* bioassay by sing the leaves containing early stages nymphal and the data were recorded after 1, 2, 3 and 4 days after treatment (Sabbour, 2013). The grasshopper, *Heteracris littoralis* (R.) is considered one of the most harmful pests to different cultivated crops in Egypt. Its economic importance comes from attacking many cultivated crops, vegetables and even trees, feeding on it and causing great losses in quantity and quality of the attacked crop. In some cases, thousands of cultivated hectares may be attacked by the swarms of grasshoppers leaving it as a divested desert. The economic injury of *H. littoralis* in Egypt had been documented by Mistikawy (1929) and Nakhla (1957) El-Shazly, (1991) Ohabuike, (1979), Ghazawy (2007). The development of suitable artificial diets for maintaining laboratory colonies of insects became of great importance for facilitating different investigations of the biology and behavior of insect, especially, if the green plants were not available. Till now, there is no published data about artificial rearing of *H. littoralis* in the laboratory, hence, the present research is an attempt for testing semi artificial diets with different additives in rearing *Heteracris littoralis* compared with green clover plant as a natural diet. The main aim of the present research is to evaluate the destruxin effect of on the grasshopper, *H. littoralis* under laboratory and semi-field condition for disrupting growth and development of *H. littoralis.*

MATERIALS AND METHODS

Insect culture.

Heteracris littoralis grasshopper was reared under laboratory condition for several generations on semi-artificial diet as mentioned by Sharaby *et al*. (2010).

Preparation of the semi-artificial diet:

The components with exception of agar were blended with water. The agar was separately dissolved in water at 100°C, cooled to 60°C and then mixed with other blended ingredients. The diet was poured in plastic cups, leaved at room temperature for solidification and then kept in the refrigeration till using nymphal period, longevity of both males and females, pre oviposit ion period, oviposit ion period, post oviposit ion period, fecundity of females and percent of egg hatchability besides life span of both males and females Sharaby *et al.* (2010).

Entomopathogenic Fungus:

The fungus, *Metarhizium anisopliae* isolated were obtained in a series of soil screening experiments by using 200 samples (Ghanbary *et al.,* 2009). 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 shake vigorously 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°C for further examinations. Bioassays: The fresh citrus leaves containing CLM larvae were collected daily and the ones including at least 10 early stages larvae used in experiments. The rate of 10 larvae per leaf was provided by killing the additional larvae before treatment. The extracted destruxin and 10, 15 and 20-fold dilutions were used in bioassays. The prepared leaves were dipped in concentrations for 10 sec and allowed to dry for about one hour. The treated leaves were placed in Petri dishes and held in incubator conditions (27±1°C). The bioassays were replicated four times for any isolate and dilution and the control was containing only distilled water. Mortality was recorded at 1, 2, 3 and 4 days after treatment. The nymphs with no movement were recorded as died ones. The probit and T-test options of SPSS software were used for analyzing time-mortality and comprising means of mortality, respectively. The percentages of mortality were calculated after seven days and corrected according to Abbott (1925), while LC_{50} s was calculated through probit analysis of Finney (1964).

Preparation of the nano-destruxin.

The extracted destruxin were prepared to nano-particles by national research center microbiological team according to Leiderer *et al.* (2008).Then prepared for scanning microscopy.

RESULTS AND DISCUSSIONS

Data in table show under laboratory conditions, the LC50 of newly hatched nymphal stage recorded 200 $X10^4$ spores/ml and 129 X $X10^4$ spores/ml. For last nymphal state the LC50s recoded 214X 10^4 and 258 X 10^4 spores/ml . Also, the LC_{50} of the adult female and male recorded, 233 and $257X10^4$ spores/ml and $279X10^4$ when treated with different concentrations of Destruxin and nano-destruxin, respectively. Also, under laboratory conditions , the LC_{50}s, were significantly decreased when the adult female of grasshopper *H. littoralis* treated with nao-destruxin and reached to 153X 10^4 spores/ml (Tables 1 and 2).

Under semi-field conditions, the percentage of infestations of *H. littoralis* significantly decreased to 1.0±0.3, 3±0.1, 5±3.0 and 10±2.9 individuals after treated with

nano-destruxin in 20, 50, 90 and 120 days, respectively as compared to 15.2±2.9, 39±3.5, 66±9.6 and 98±6.6 individuals in the control (Table 3).

Figure (1) shows the scanning electron microscopy of the nano destruxin particles. The same results obtained by Sabbour (2013, a & b) Sharaby *et al.* (2010 & 2011), although the mode of action of destruxins in insects is an unclear issue but altering the calcium cannels function has proposed in some investigations (Dumas *et al.*, 1996; Samuels *et al*., 1988). Primarily, tetanic paralysis is the common symptom in insects causing by application of destruxin (Samuels *et al.*,1988). Opening the Ca^{2+} channels as a result of membrane depolarization by destruxin has been implicated as a cause of paralysis and death (James *et al*.,1993). Humoral immune response seems to be specifically affected by destruxin (Pal *et al.*, 2007).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 (Lacey, 2008; Dezianianer *et al*. (2010). Thus for investigating a cost effective procedure CLM larvae were examined by serial dilutions (10, 15 and 20 folds) of destruxins (Mohammadi Sharif *et al.*, 2010). In many cases, entomopathogens are potential control agents against pests and use along with other component of integrated pest management (Kaya and Lacey, 2007). Therefore the dilutions of extracted destruxin are suitable candidates in these programs; they decrease application's cost of destruxin in an integrated procedure. (Mohammadi Sharif *et al*., 2010) reported that, 10-fold dilution of A-115 seems to be a good example of an accompanying agent in these programs. Like other microbial agents, long period of lethal infection (Lacey, 2008) is a disadvantage of destruxins. This make the LT_{50} value consider as a significance parameter. The first three instars are active and feed within the mines, while the forth instar, 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 (Beattie and Hardy, 2004 and Ghazawy, 2005), but indifferent environmental conditions it may be longs one to three weeks (Ba-Angood, 1978; Godfrey and Grafton-Cardwell, 2002; Grafton-Cardwell, 2009). By considering the biology of CLM and the LT_{50}s of isolates (Mohammadi Sharif, *et al*., 2010), destruxin have acceptable efficacy on CLM. Also, these measures suggest that three to four days after treatment is a suitable endpoint for destruxin bioassay. On the other hand, fungus contamination of the food source could repel larvae and thus reduce their food consumption, as was shown for the grasshopper *Stiphrarobusta* exposed to leaves sprayed with *M. anisopliae* var. *acridum* (Magalhaes *et al.*, 2001). Also, Mohammadi Sharif *et al*. (2010), reported that, Microbial agents are a suitable and naturally friend part of integrated pest management programs. Ibrahim (1968) found that *Vicia faba* gave the highest fecundity in *Pyrgomorpha conica*, while the same plant permitted moderate fecundity in *Chrotogonus lugubris* (Ibrahim, 1971, 1967 and 1968). The effect of food type on the female fecundity was reported also in *Schistocerca gregaria* by Manchanda *et al*. (1982); Manchanda *et al*. (1982).

Table 1. Effect of destruxin against the desert locust *H. littoralis* under laboratory conditions.

Stages	LC_{50}	V	S	95% confidence limits
Newly hatched nymphs	200	0.01	1.3	148-247
Last nymphal stage	214	0.01	0.2	200-257
Adult ♀	243	0.01	1.2	200-281
Adult ♂	257	1.01	0.2	210-300

Table 2. Effect of nano-destruxin against the desert locust *H. littoralis* under semi-field conditions.

Stages	LC50	V	S	95% confidence limits
Newly hatched nymphs	129	0.01	1.3	201-248
Last nymphal stage	158	0.01	0.1	210-279
Adult ♀	179	0.01	1.1	200-297
Adult ♂	153	1.00	0.1	230-358

Table (3): Effect of destruxin against *H. Littoralis* under semi field conditions

treatments	Days after treatment	No .of infestations of the target pests (Means ± S.E.)
control	20	15.2±2.9
	50	39±3.5
	90	66±9.6
	120	98±6.6
Nano-destruxin	20	1.0±0.3
	50	3±0.1
	90	5±3.0
	120	10±2.9

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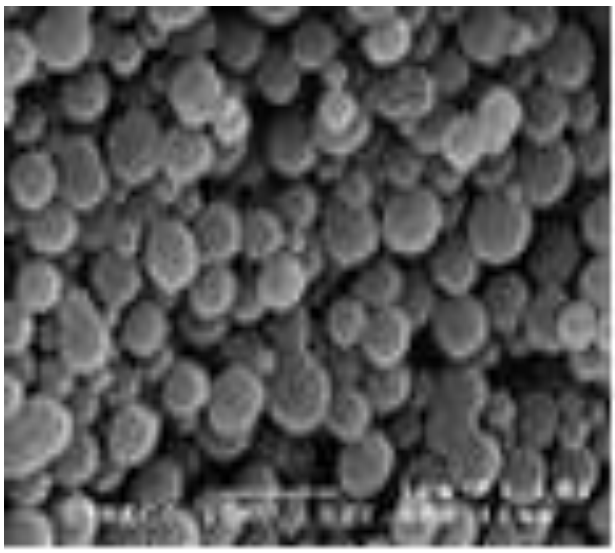

Fig. 1. Nano destruxin scanning electron microscopy.

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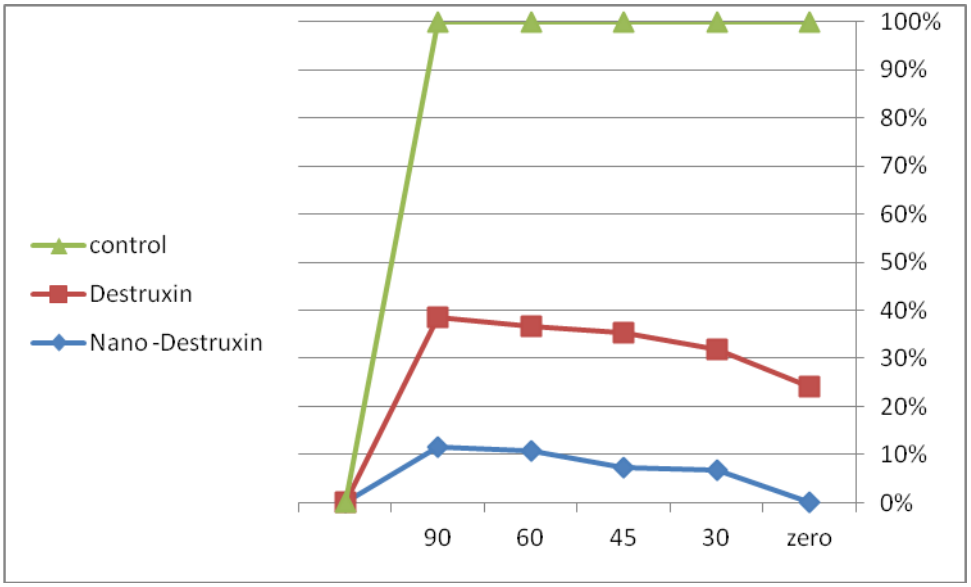

Fig. 2: percentage of infestations of the grasshopper under laboratory conditions after treatments.

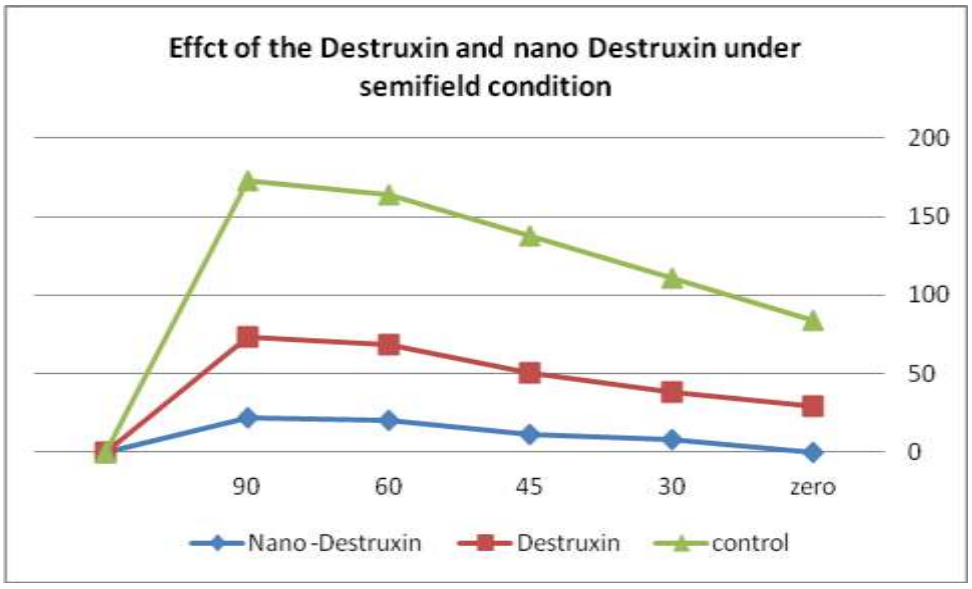

Fig3. Effect of destruxin and nano-destruxin against grasshopper under semi field Condition.

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تقييم سمية النانو ديستروكسين المستخرج من الميتاريزيوم انيزوبيلا ضد النطاط *Hetiracris littoralis* فى مصر

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الفطر ميتاريزيوم انيزوبيلا (Metschnikoff) سوركين ينتج بعض السموم الببتدية الحلقية و الدستروكسين الذى يثبط تأثير فعل المبيد . يعتبر النطاط *Hetiracris littoralis* آفة رئيسية تأكل النباتات الخضراء و تسبب فى الكثير من الإصابات ، لذلك المكافحة الآمنة المؤثرة تعتبر موضوع هام . الفطر المعزول ميتاريزيوم انيزوبيلا ينمى عل الميديا Potato Dextrose Broth (PDB) mediums**.** و قد إستخرج النانو ديستروكسين بواسطة إضافة الكلوروفورم . وايضا قد عوملت الأوراق التى تحتوى علية قبل تغذية المراحل العمرية الصغيرة من النطاط *Hetiracris littoralis* . و اخذت النتائج التى دلت تحت ظروف المعمل ان LC50s جرثومة/مل سجلت 200 X10^4 spores/ml and 129 X X10^4 **جرثومة/مل** للحوريات حديثة الفقس و الحوريات ثم الطور اليافع بالترتيب. و ايضا اثبتت النتائج ان LC50s للطور اليافع هو للأنثى و الذكر 279X10^4 , 233 ، 257X10^4 جرثومة/مل *بالترتيب. و ايضا اثبتت* التجارب ان عدد الإصابة بالنطاط قد فلت معنويا بعد استخدام النانو ديستركسين

تحت الظروف الشبة حقلية دلت النتائج على ان نسبة الإصابة بالنطاط قد قلت معنويا بعد المعاملة بالنانو ديسركسين إلى 1.0±0.3, 3±0.1, 5±3.0 and 10±2.9 فردا بعد 20 ، 50 ، 90 ، 120 يومابالترتيب مقارنة بالغير معامل 15.2±2.9, 39±3.5, 66±9.6 and 98±6.6 فردا فة نهاية التجربة.