

Evaluating toxicity of extracted nano -Destruxin against the desert locust *Schistocerca gregaria* in Egypt

Magda M. Sabbour

Pests and Plant Protection Dep., National Research Centre ,Dokki, Cairo Egypt. El- Behouth St.,
P.O. Box 12622, Dokki, Cairo, Egypt
E-mail: sabbourm9@yahoo.com

ABSTRACT

Nano destruxin tested under laboratory and semifield condition. Results show that the LC_{50} s of the locust *S. gregaria* after treatment with destruxin , 210×10^4 , 221×10^4 , 250×10^4 spores/ml, of newly hatched nymphs last nymphal stage and adult stage., respectively. The results show that, the LC_{50} s of the corresponding stages significantly decreased after treated with the nano-destruxin which is recode, 99×10^4 , 106×10^4 and 114×10^4 spores/ml . Under semi field condition, the LC_{50} s of newly hatched nymphs, last nymphal stage and adult stages, 210×10^4 , 227×10^4 and 224×10^4 spores/ml .

The effect of nano-destruxin against *S. gregaria* under semi-field conditions show that after 20 days, the infestations number were significantly decreased to 2.2 ± 1.2 , as compared to 2.4 ± 5.3 , and 12.2 ± 2.2 individuals after treated with destruxin and in the control. After 90 days of the post applications, the corresponding infestations, recorded that, 20 ± 8.9 , 18 ± 3.4 and 60 ± 4.9 individuals, respectively. After 120 days the infestations number significantly decreased to 29 ± 8.9 individuals after treated with nano-destruxin.

Key words: Destruxin, *Metarhizium anisopliae*, *Schistocerca gregaria*, microbial agent.

INTRODUCTION

Nanotechnology is a promising field of interdisciplinary research. It opens up a wide array of opportunities in various fields like medicine, pharmaceuticals, electronics and agriculture. The potential uses and benefits of nanotechnology are enormous. These include insect pests management through the formulations of nanomaterials-based pesticides and insecticides, enhancement of agricultural productivity using bio-conjugated nanoparticles (encapsulation) for slow release of nutrients and water, nanoparticle-mediated. Desert locust *Schistocerca gregaria* bioassayed by using the nano destruxin leaves containing early stages larvae. Sabbour (2013) showed that range of mortality was between 64-85% based on the end point data. Bioassay of destruxin by against desert locust *Schistocerca gregaria* nymphs showed its acceptable effect of destruxin. By considering biology of this species and calculated LC_{50} s, destruxin seems to be effectiveness.

The life history of the desert locust, *Schistocerca gregaria* (Forsk.), and the epidemiology of its outbreaks were obscure until the 20th Century. One of the discoveries of importance in this century was that of solitary and gregarious phases. A most prominent feature of the 'arbeh' is that it can exist as scattered individuals within the 'recession areas' or, when gregarious, as swarms throughout the 'invasion areas'. This is because the locust exists in different phases. When breeding conditions lead to an increase in the number of locusts crowded together, the

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insects have the ability to change their color, behavior, shape and physiology, with color and behavior being the characteristics to change first.

All grasshoppers (except for the so-called long-horned grasshoppers) belong to the superfamily Acridoidea; the most important locusts are all in the family Acrididae. The extensive devastation caused by insect pests to crops and pasture lands since antiquity have left a profound impact on historical records from all over the ancient Middle East. One of the best known examples is the vivid description of a desert locust invasion by the prophet Joel in the Bible, referring to the ravages of the huge swarms of hoppers. Of the ten plagues which, according to the Old Testament, the Lord inflicted on Pharaoh's Egypt, three were insect plagues, namely, lice, flies and locusts.

Egypt, from 4500 years ago (Amer *et al.*, 2008; Uvarov, 1977 and 1966). More than 300 species of locusts and grasshoppers are known to exist in the African continent (FAO (1995)), but fortunately only a few of them are major pests; most are sedentary, inhabiting a rather confined area throughout their life cycle. Only few species are highly mobile, migrating from one location to another with ecological conditions more suitable for feeding and reproduction. The desert locust in Africa exhibits fully gregarious characteristics and is known for its long-range pattern of migration, covering as many as several thousand kilometers within a single generation. This is the main reason why the locust was, not surprisingly, the most feared among agricultural pests in antiquity. Although our knowledge regarding plant protection problems in antiquity in the Near East is generally limited, some sources, such as the Biblical and Rabbinical (Halakha and Midrash) literature, go into more detail when referring to the desert locust. Greek and Roman literature also deal extensively with various aspects of 'a kris' and 'locusta', (for the terms of these locusts and other pests belonging to the order Orthoptera), respectively. Under such conditions not only the flora and fauna are multifarious, but also their pathogens, predators and parasites, which include all sorts of animals – invertebrates as well as vertebrates, and lower and higher plants. It may be assumed that proliferation of the pathogens and parasites was often counterbalanced in ancient times by natural enemies. It is not clear whether this applies also to locusts.

The aim of this work is to evaluate the toxicity of extracted nano-destruxin from the fungus *Metarhizium anisopliae* against the desert locust *Schistocerca gregaria* in Egypt.

MATERIALS AND METHODS

Insect rearing

Locust was reared under laboratory condition for several generations on semi-artificial diet as mentioned by 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

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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 ($27\pm 1^\circ\text{C}$). 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 larvae 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} 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 DISCUSSION

Data in Table (1) show that the LC_{50} s of the locust *S. gregaria* after treated with destruxin were 210×10^4 , 221×10^4 , 250×10^4 spores/ml. of newly hatched nymphs last nymphal stage and adult stage, respectively. Table (2) shows the LC_{50} s of the corresponding stage significantly decreased after treated with the nano-destruxin which is recode, 99×10^4 , 106×10^4 and 114×10^4 spores/ml .

Under semi field condition, the LC_{50} s of newly hatched nymphs, last nymphal stage and adult stages were 210×10^4 , 227×10^4 and 224×10^4 spores/ml, respectively (Table 3).

The effect of nano-destruxin against *S. gregaria* under semi-field conditions show that after 20 days, the infestations number was significantly decreased to 2.2 ± 1.2 . As compared to 2.4 ± 5.3 , and 12.2 ± 2.2 individuals after treatment with destruxin and in the control, respectively (Table 4). After 90 days of the post applications, the corresponding infestations, recorded were 20 ± 8.9 , 18 ± 3.4 and 60 ± 4.9 individuals , respectively. After 120 days the infestations number significantly decreased to 29 ± 8.9 individuals after treated with nano-destruxin (Table 4).

Figure (1) shows the nano destruxin particles with scanning electron microscopy. Figure (2) shows that the laboratory experiments indicated that the nano-destruxin is the most effective against the locust *S. gregaria* comparing with the destruxin and the control. Figure (3) shows that the infestations of the locust *S. gregaria* under semi field conditions comparing the destruxin and non –destruxin which show that the infestation percent significantly decreased after treated with nano –destruxin.

The entomopathogenic fungus *Metarhizium anisopliae* is a well studied and applied species for microbial control of insect pests (Liu *et al.*, 2007; Hoe *et al.*, 2009). This fungus produces some cyclic peptides, destruxins (Hsiao and Ko, 2001) which may play a role in its pathogenicity (Kershaw *et al.*, 1999). More than 35 different structurally related destruxins have been isolated from cultures of *M. anisopliae* (Pedras *et al.*, 2002). Destruxin capacity in control practices is a less studied matter, but some investigations have described its insecticidal properties (Brousseau *et al.*, 1996; Thomsen and Eilenberg, 2000). Sabbour (2013a and b) examined the effect of different dilutions of destruxins extracted from several fungal isolates on desert locust.

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Although the mode of action of destruxins in insects is an unclear issue but altering the calcium channels 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 the present 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; Dezanian *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 fourth 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), but in different environmental conditions it may be long 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 *Stiphra robusta* 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. Extracted destruxin from the fungi, *M. anisopliae* can be used as a constituent of citrus leafminer control measures. Like many of microbial agents, the slow acting property of destruxin should be considered in citrus leafminer measures. However, before it is used as a component of an IPM procedure, its compatibility with other biocontrol agents such as beneficial insects and the pesticides used in citrus orchards has to be examined.

Table 1. Effect of destruxin against the desert locust *S. gregaria* under laboratory conditions.

Stages	LC_{50}	V	S	95% confidence limits
Newly hatched nymphs	210×10^4	0.01	1.3	201-227
Last nymphal stage	221×10^4	0.01	0.2	211-277
Adult	250×10^4	0.01	1.2	222-289

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Table 2. Effect of nano-destruxin against the desert locust *S. gregaria* under laboratory conditions.

Stages	LC ₅₀	V	S	95% confidence limits
Newly hatched nymphs	99 X 10 ⁴	0.01	1.3	88-227
Last nymphal stage	106 X 10 ⁴	0.02	0.2	97-277
Adult	114 X 10 ⁴	0.03	1.2	99-289

Table 3. Effect of nano-destruxin against the desert locust *S. gregaria* under semi-field conditions.

Stages	LC ₅₀	V	S	95% confidence limits
Newly hatched nymphs	210 X 10 ⁴	0.01	1.3	200-247
Last nymphal stage	227 X 10 ⁴	0.01	0.1	210-279
Adult	244 X 10 ⁴	0.01	1.1	202-299

Table (4): Effect of nano-destruxin against *S. gregaria* under semi-field conditions.

Treatments	Days after treatment	infestations of the target pests
Control	20	12.2±2.2
	50	29±3.9
	90	60±4.9
	120	88±6.9
Destruxin	20	2.4±5.3
	50	10±4.4
	90	18±3.4
	120	24±2.9
Nano- destruxin	20	2.2±1.2
	50	19±3.9
	90	20±8.9
	120	29±8.9

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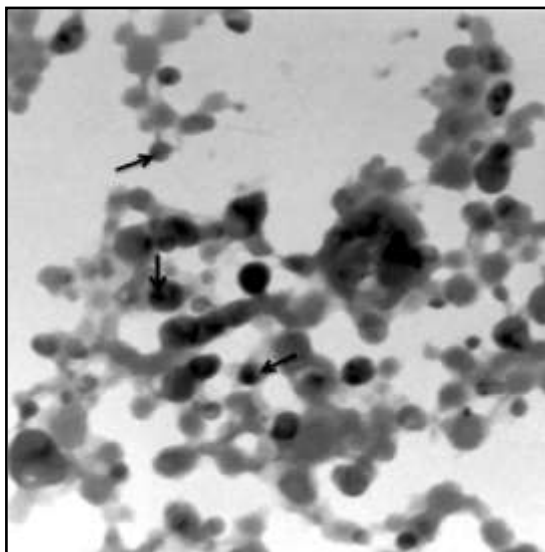


Fig.1. Nano destruxin scanning electron microscopy.

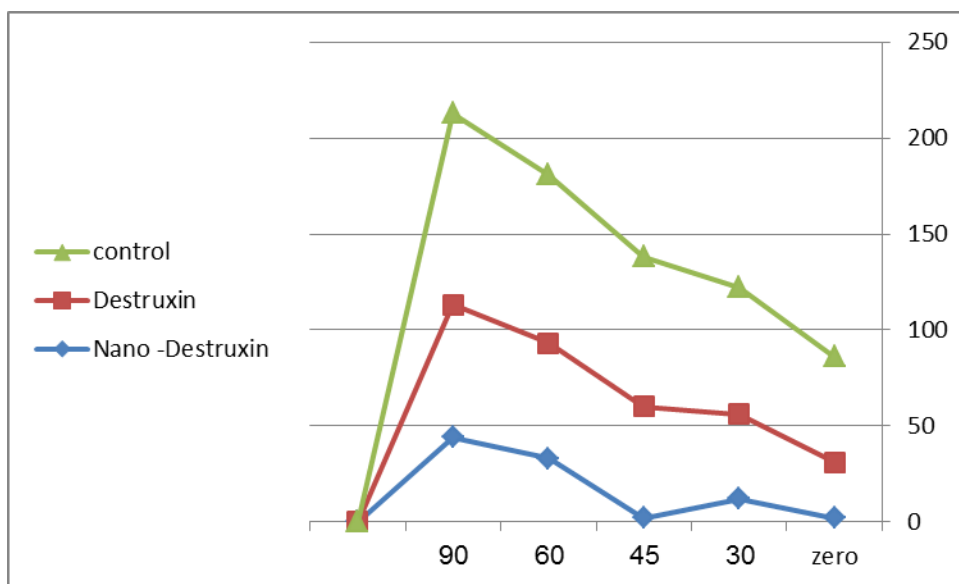


Fig . 2: Percentage of infestations of the locust *S. gregaria* under laboratory conditions after treatments

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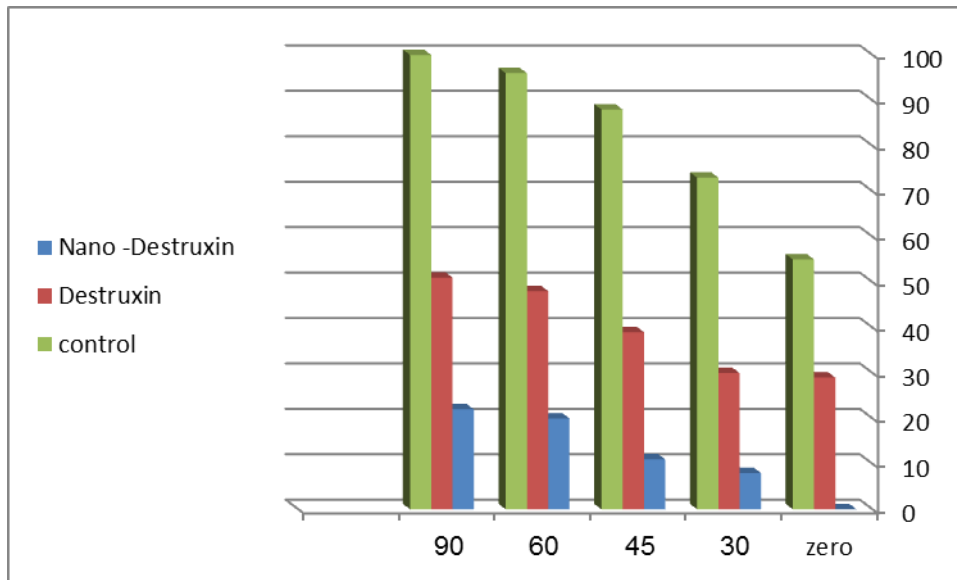


Fig. 3: Percentage of locust infestations percentage of infestations of the locust *S. gregaria* under semi field conditions after treatments.

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تقييم سمية النانو ديستروكسين المستخرج من المياتريزيوم انيزوبيللا ضد الجراد الصحراوي
Schistocerca gregaria في مصر

ماجدة صبور
قسم آفات ووقاية النبات - الشعبة الزراعية - المركز القومي للبحوث
ش التحرير الدقي - القاهرة
E-mail: sabbourm9@yahoo.com

المستخلص

الفطر ميتاريزيوم انيزوبيللا (Metschnikoff) سوركين ينتج بعض السموم واليبتيدات الفطرية الديستروكسين التي تثبط تأثير المبيدات , يعتبر الجراد الصحراوي أفة ضارة تأكل النباتات الخضراء بشراهة وتسبب الكثير من التلف للنباتات وبالتالي يجب السيطرة الفعالة وأمنة هو مسألة هامة. تم استخراج الفطر وتنميتها على الميديا Potato Dextrose Broth (PDB) mediums وإضافة الكلوروفورم إليها. وتم عمل الديستروكسين في هيئة نانو ومعاملة الجراد الصحراوي بها وقد اظهرت النتائج وبعد معاملة المراحل الأولية للأفة الجراد الصحراوي بعد تغذيتها على الأوراق المعاملة بالتركيزات المختلفة للنانة ديستروكسين تحت ظروف المعمل والحقل أن ال LC50s للجراد الصحراوي هو 210×10^4 , 221×210 10^4 , 250×10^4 جرثومة/مل للحوريات حديثة الفقس و الحوريات ثم الطور اليافع بالترتيب. وكذلك بينت النتائج أن LC50s لنفس الأعمار السابقة [قلت معنويا بعد معاملتها بالنانو ديستروكسين و سجلت : 99×10^4 , 106×10^4 , 114×10^4 جرثومة/مل. و قد دلت النتائج انه تحت ظروف الشبهة حقلية أن LC50s هي: 210×10^4 , 227×10^4 جرثومة/مل للحوريات حديثة الفقس والحوريات ثم الطور اليافع بالترتيب . وقد دلت أيضا أن بعد 20 يوما فإن نسبة الإصابة قلت معنويا الى 2.2 ± 1.2 مقارنة 2.4 ± 5.3 , 12.2 ± 2.2 بالغير معامل بالترتيب. وبعد 90 يوما من المعاملة بالنانو ديستروكسين للجراد الصحراوي قد دلت النتائج ان عدد الأفراد قد قلت معنويا الى 18 ± 3.4 , 20 ± 8.9 , 60 ± 4.9 فردا بالترتيب. و بعد 120 يوما من المعاملة للجراد الصحراوي بالنانو ديستروكسين قد قلت أعداد معاملة الأفراد .