

# Novel Determinations of Nano-extracted Destruxin from *Metarhizium anisopliae* against *Ephestia cautella* and *Ephestia Kuehniella* (Lepidoptera-Pyralidae) under Laboratory and Store Conditions

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**Abstract:** The Destruxin is a cyclic hexadepsipeptides produced by entomopathogenic and phytopathogenic fungi five amino acids and one hydroxyl acid. The effect of Destruxin and nano-Destruxin on the red flour beetle -*Tribolium castaneum* and confused flour beetle -*Ephestia Kuehniella* (Lepidoptera-Pyralidae), under laboratory and store conditions were studied. The half life period LC50 of *Ephestia cautella* when treated with different concentrations of the fungi toxin show recorded.  $111 \times 10^4$  and  $46 \times 10^4$  spores / ml for destruxin and nano- destruxin treatments. The half life period LC50 of *E. Kuehniella* recorded that,  $121 \times 10^4$  and  $32 \times 10^4$  spores/ ml after destruxin and nano-destruxin treatments. Also, results, show the effect of both toxin examined against *E. cautella* biology, detected that, the number of eggs laid /female obtained were significantly decreased to  $50 \pm 1.7$  and  $28 \pm 3.9$  eggs/female after destruxin and nano-destruxin treatments as compared to  $369 \pm 8.1$  eggs/ female in the control.

**Keywords:** *Ephestia cautella*, *Ephestia Kuehniella*, Destruxin, nano- Destruxin

## 1. Introduction

The Destruxin is a cyclic hexadepsipeptides produced by entomopathogenic fungus and phytopathogenic fungi five amino acids and one hydroxyl acid. *Ephestia cautella* is an important insect in store worldwide as pest of stored products that is observed among several commodities. This pest may cause considerable economical losses if not adequately controlled because it has a very high rate of population increase [1]. Isolates of *M. anisopliae* were inoculated in Potato Dextrose Broth (PDB) mediums. Destruxin was extracted by adding chloroform. Usage of bioinsecticid on Grasshopper *Heteracris littoralis* bioassay by sing the leaves containing early stages nymphal and the data were recorded after 1, 2, 3 and 4 days after treatment [2]. 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.[2] 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<sub>50</sub>s, destruxin seems to be an effectiveness.

The Mediterranean Flour Moth, Indian Flour Moth or Mill Moth (*Ephestia kuehniella*) and The almond moth or tropical warehouse moth *Ephestia cautella* (Lepidoptera-Pyralidae), Caterpillars produce a lot of webbed galleries and join the grains together and also contaminate food items with faecal matter. Webbing of grains produces lumps as in the case of *Corcyra*. In heavy infestation the surface of the entire stock

can be covered by silken webs left behind by the wandering larvae. [4]. "Mediterranean Flour Moth". Penn State College of Agricultural Sciences. Retrieved 2013-09-07The caterpillars are often found feeding on flour, cereals, baked goods and other dry grain products in food storage areas.[1] Less often, dried fruits or mushrooms and even peat or rotting wood may be eaten. The species may reach extreme population densities in suitable locations (such as gristmills) if left uncontrolled, and the silken webs produced by the caterpillars may even interfere with normal operations of machinery such as flour sieves.[3] The adult moths do not feed [4, 5]. This work aims to evaluate the nano destruxin against *Ephestia cautella* and *Ephestia Kuehniella*

## 2. Materials and Methods

### Tested Insects

Larvae of *E. cautella* and *E. Kuehniella* were used in the experiments. The target insects were reared under laboratory conditions on semi-artificial diet (fine wheat with some adherent endosperm) with 20% glycin and 5% yeast powder. All cultures and experiments were held at  $26 \pm 2$  °C and 70-80% R.H. with 16 hours light and 8 hours dark.

### Preparation of the nano-destruxin.

The extracted destruxin were prepared to nano-particles by national research centre microbiological team according to [6]. Then prepared for scanning microscopy.

### Bioassays

The insecticidal efficacy of nano-destruxin was tested at three dose rates, 0.25, 0.50 and 1 g/kg wheat against the 3<sup>rd</sup> instar larvae of *E. cautella* and *E. Kuehniella* (Lepidoptera-Pyralidae). For each case, four glass jars as replicates were used. Each replicate was treated individually with the respective nano-destruxin quantity and then shaken manually for one minute to achieve equal distribution of the nano-

destruxin. Subsequently, ten 3<sup>rd</sup> instar larvae of the two tested species were introduced into each glass jar and covered with muslin for sufficient ventilation. Twelve replicates glass jars containing untreated wheat served as control. Mortality was assessed after 7 d of exposure in the treated and untreated jars. Mortality was corrected according to [7]. All tests were conducted at 27 ± 2 °C and 65 ± 5% relative humidity (RH). All the experiments were repeated three times. The nano-destruxin. Destruxin were used at the rate of 0.5 g/kg wheat. Four replicates of 100 g wheat for each treatment were used. Each replicate was treated individually with the formulations for 1 min and put inside glass jars. Four replicates in jars containing untreated wheat served as control. Subsequently, one paired of newly emerged adults were introduced into each jar. The number of deposited eggs on treated or untreated wheat/female was counted and the percent repellence values were calculated according to the equation of [8] ,  $D = (1 - T/C) \times 100$ , where: T and C represent the mean number of deposited eggs per female of the treated and check set, respectively. Four replicates jar containing untreated grain served as control. Subsequently, one paired of newly emerged adults were introduced into each jar. The number of deposited eggs on treated or untreated grains/female was counted. The data was analyzed using analysis of variance (ANOVA), where significant differences between the treatments were observed. Mean values were significantly separated by using the least significant difference (LSD) test at 5% level.

### 3. Results and Discussions

Table 1 show that the half life period LC50 of *Ephestia cautella* when treated with different concentrations of the fungi toxin. The results show that LC50 111X10<sup>4</sup> and 46X10<sup>4</sup> spores / ml for destruxin and nano- destruxin treatments. The half life period Lc50 of *Ephestia Kuehniella* recorded that, 121X10<sup>4</sup> and 32 X10<sup>4</sup> spores/ ml after destruxin and nano-destruxin treatments (Table2).

Table 3, show the effect of both toxin examined against *Ephestia cautella* different stages of life cycle, the results show that the number of eggs laid /female obtained were significantly decreased to 50 ± 1.7 and 28±3.9 eggs/female after destruxin and nano-destruxin treatments as compared to 369±8.1 eggs/ female in the control (Table 3).

Figure 1, show the nano-particles of the toxin which measure 200nm of nano- destruxin. Figure 2show under store conditions the infestations of *Ephestia cautella* were significantly decreased as compared to control.

Figure3, show the infestations percentage of *Ephestia Kuehniella* which show that he nano- destruxin scored a highly effect in decreasing the infestation number after 100

days of treatments in the store. The same results meet with [9, 10,11] tested the insecticidal effects of modified DE with different hydroxides against *C. maculatus* on stored cowpea grains. These results meet with [12,13,14,15, 16] who found that in all tested insects there were significant differences between DEs alone compared to untreated control. The combination of Ca-DE and Na-DE with tested fungi highly suppressed the moths' egg production in comparison to untreated with highly significant differences. A moderate effect on suppressing the moths' egg production was recorded in case of DE and Al-DE with tested fungi. *P. interpunctella* was the most susceptible moth to DE/fungi combinations followed by *E. cautella* and *E. kuehniella*. The application of Ca-DE caused the complete mortality of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) compared to the other tested DEs after 7 and 14 d interval [9,10].The presence of DE fevers the insecticidal efficacy of *Beauveria bassiana* (Balsamo) *Vuillemin* (Ascomycota: Hypocreales) against larvae of *R. dominica*. The addition of many inert dust types such as charcoal, ash or DE increased the potency of *M. anisopliae* against *S. Oryzae*[11] . [10] mentioned that DE significantly increased the attachment of *B. bassiana* conidia on the cuticle of *T. castaneum* larvae. This attachment resulted to damage the epicuticle lipids of insects (Moore et al., 2000) tested the effect of four fungal isolates, (*B. bassiana*, *L. lecanii*, *M. anisopliae* var. *anisopliae* and *I. farinosa*) on adults of *P. interpunctella* and one species tested on mature larvae of the pest. The same results obtained by [17, 18, 19 ].

[9] found that the nano particles of aluminium oxide and titanium oxide highly reduced the infestations of *Sitophilus oryzae* under laboratory and store conditions. [2]Sabbour, 2013a, proved that of Nanoparticle (silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500) were more effective in controlling *S. oryzae* than of (silica gel Cab-O-Sil-750, silica gel Cab-O-Sil-500) alone. [3], determined that Nano-particle (Zinc oxide ZnO) more effective in decreasing the infestation with *S. oryzae* under laboratory and sore condition. Sabbour, [1, 2, 3] used the nano Destruxin against the locust and grasshopper which proved that the nano materials decreased the infestation number of both pests.

**Table 1:** Effect of the pathogen against *Ephestia cautella*

Target pathogen	LC <sub>50</sub>	S	V	95% Confidence limits
Destruxin	111X10 <sup>4</sup>	0.1	1.4	79-109
nano- Destruxin	46X10 <sup>4</sup>	1.1	1.1	37-99

**Table 2:** Effect of the pathogen against *Ephestia Kuehniella*

Target pathogen	LC <sub>50</sub>	S	V	95% Confidence limits
Destruxin	121X10 <sup>4</sup>	0.1	1.4	81-137
nano- Destruxin	32 X10 <sup>4</sup>	1.1	1.1	17-59

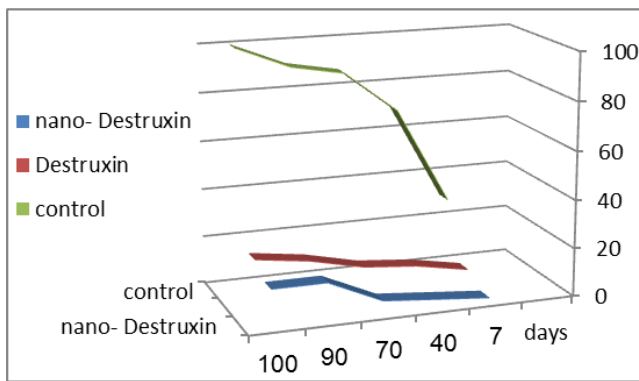
**Table 3:** Effect of the tested toxins on the *Ephestia cautella* biology

Target pest	No of eggs laid/female	% of egg hatching	% of larval mortality	% of malformed larvae	% of malformed pupae	% of emerged adults	% of malformed adults
Destruxin	50±1.7	4	77	76	77	3	79
nano- Destruxin	28±3.9	2	89	89	79	30	79
Control	369±8.1	100	-	-	-	100	-
F value	31.0	2	5	5	22	21	20
Lsd5%	11.1	2	3	3	11	11	9

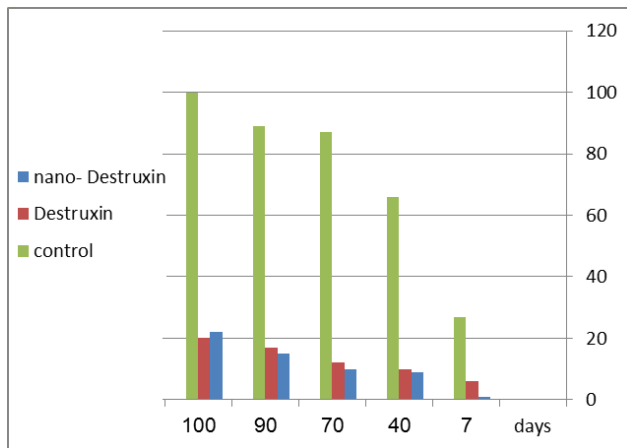
**Table 4:** Effect of different toxins treatments on *E. Kuehniella* under store conditions

Storage interval days	Control		Destruxin		nano- Destruxin	
	No of eggs laid/female	% adult Emergence (F1)	No of eggs laid/female	% adult Emergence (F1)	No of eggs laid/female	% adult Emergence (F1)
20	11.8±7.5	87	8.1±2.5	14	1.8±3.9	2
45	51.8±6.5	95	18.4±9.5	2	7.7±1.8	3
90	96.1±3.5	98	30.1±2.5	34	10.1±4.5	10
120	99.1±1.8	100	51.8±6.9	56	19.1±1.5	10
F value	20.1	20.6	10.3	23.3	20.1	10.1
Lsd5%	10	12	11	Lsd5%	11	7

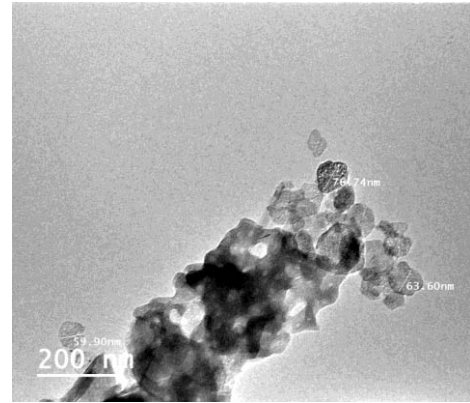
The effect of the destruxin and nano-destruxin on *Ephestia Kuehniella* infestations under store conditions show that, the number of eggs laid/female were significantly decreased to 51.8±6.9 and 19.1±1.5 eggs/female when treated with destruxin and nano-destruxin, respectively after 120 days of storage treatments as compared to 99.1±1.8 eggs/female in the control (Table4).



**Figure 2:** Infestation percentages of *Ephestia cautella* under store conditions



**Figure 3:** Infestation percentages of *Ephestia Kuehniella* under store conditions



**Figure 1:** Scanning electron microscopy nano- Destruxin

## References

- [1] Sabbour, M.M. 2014. Efficacy of some microbial control agents and inorganic insecticides against red flour beetle *Tribolium castaneum* and confused flour beetle, *Tribolium confusum* (Coleoptera: Tenebrionidae) Integrated Protection of Stored Products. IOBC-WPRS Bulletin Vol. 98, 2014.pp. 193-201.
- [2] Sabbour M.M. & Abdel-Rahman. 2013. Efficacy of isolated *Nomuraea rileyi* and Spinosad against corn pests under laboratory and field conditions in Egypt Annual review and research in biology, 3(4): 903-912.
- [3] Sabbour, M.M., A.A. Abd-El-Rahman & M.A. Ragei. 2013. Determinations of some extracted oils in controlling two stored product insect pests. Middle East Journal of Agriculture Research, Middle East Journal of Agriculture Research, 2(4): 127-132, 2013
- [4] Steve Jacobs and Dennis Calvin (October 1988). "Mediterranean Flour Moth". Penn State College of Agricultural Sciences. Retrieved 2013-09-07.
- [5] Sabbour M.M. & M.A. AbdEl-Raheem. 2013. Repellent Effects of *Jatropha curcas*, canola and Jojoba Seed oil, against *Callosobruchus maculatus* (F.) and *Callosobruchus schinensis* (L.). Journal of Applied Sciences Research, 9(8): 4678-4682, 2013
- [6] Leiderer, P. and Dekorsy, T. (2008). Interactions of nanoparticles and surfaces Tag der mÄundlichenPrÄufung: 25. April. URL: <http://www.ub.unikonstanz.de/kops/volltexte/2008/5387/>;
- [7] Abbott, W. S. 1925: A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.
- [8] Lwande, W., Hassanalli, A., Njorage, P. W., Bentley, M. D., DelleMonache, F. & Jondiks, J. I. 1985: A new 6a-hydroxy petrocarpan with insect antifeedant and

- antifungal properties from the root of *Tephrosia hildebrandtu*. *Vatle. Ins. Sci. Appl.* 6: 537-541.
- [9] Abd-El-Aziz, S. E. & Sherief, M. A. 2010. Insecticidal effects of modified diatomaceous earth (DE) with different hydroxides (MOH, M = Na, Ca, Al) against *Callosobruchus maculatus* (F.) beetles (Coleoptera: Bruchidae) on stored cowpea grains. *J. Entomol. Res.* 34: 1-9.
- [10] Akbar, W., Lord, J. C., Nechols, J. R. & Howard, R. W. 2004: Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. *J. Econ. Entomol.* 97: 273-280.
- [11] Batta, Y. A. 2004: Control of the rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*. *Crop Prot.* 23: 103-108.
- [12] Sabbour, M. M. & Sahab, A. F. 2005: Efficacy of some microbial control agents against cabbage pests in Egypt. *Pak. J. Biol. Sci.* 10: 1351-1356.
- [13] Sabbour, M. M. & Abd El-Aziz, S. E. 2007a: Efficiency of some bioinsecticides against broad bean beetle, *Bruchus rufimanus* (Coleoptera: Bruchidae). *Res. J. Agric. Biol. Sci.* 3: 67-72.
- [14] Sabbour, M. M. & Abd-El-Aziz, S. E. 2007b: Evaluation of some bioinsecticides and packaging materials for protecting broad bean against *Callosobruchus maculatus* (Coleoptera: Bruchidae) infestation during storage. *Bull. Ent. Soc. Egypt* 33: 129-137.
- [15] Sabbour, M. M. & Abd-El-Aziz, S. E. 2010: Efficacy of some bioinsecticides against *Bruchidius incarnates* (Boh.) (Coleoptera: Bruchidae) infestation during storage. *J. Plant Prot. Res.* 50: 25-31.
- [16] Sabbour, M. M. & Abd-El-Aziz, S. E., Sherif, M. A. 2012: Efficacy of three entomopathogenic fungi alone or in combination with diatomaceous earth modifications for the control of three pyralid moths in stored grains. *J. Plant Prot. Res.* 52: 359-363.
- [17] Sabbour, M.M. & Shadia E. Abed El-Aziz. 2002. Efficacy of some botanical oils formulated with microbial agents against the cotton leafworm and greasy cutworm attacking cotton plants. *Bull. ENT.Soc. Egypt.* 2002: 5(28): 135-151.
- [18] Kavallieratos, N. G., Athanassiou, C. G., Vayias, B. J. & Maistrou, S. N. 2007a: Influence of temperature on susceptibility of *Tribolium confusum* (Coleoptera: Tenebrionidae) populations to three modified diatomaceous earth formulations. *Fla Entomol.* 90: 616-625.
- [19] Kavallieratos, N. G., Athanassiou, C. G., Mpakou, F. D. & Mpassoukou, A. E. 2007 b: Factors affecting laboratory bioassays with diatomaceous earth on stored wheat. Effect of insect density, grain quantity, and cracked kernel containment. *J. Econ. Entomol.* 100: 1724-1731. 200 .
- [20] Kavallieratos, N. G., Athanassiou, C. G., Pashalidou, F. G., Andris, N. S. & Tomanović, Ž. 2005: Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). *Pest Manag. Sci.* 61: 660-666.