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Influence of *Metarhizium anisopliae*-based Destruxin A-760 and Destruxin - A-724 on the sugar beet fly, *Pegomya mixta* Vill. (Diptera: Anthomyiidae)

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ABSTRACT

Sugar beet contains about 15-20% sugar is considered a very important economic crop which produces the sugar in all worlds and also in Egypt. The sugar beet fly, *Pegomya mixta* Vill. belongs to (Diptera: Anthomyiidae), causing a damage to sugar beet crop plantations causing a blotch or "blister". *Metarhizium anisopliae* is entomopathogenic fungus produce toxin called Destruxin (DEX). This toxin could to paralysis the insects, also cause pests death. Destruxin 760and Destruxin A-724 examined in the laboratory by six concentrations. the lethal concentration LC_{50} was 76 and 141 ppm when sugar beet fly, *P. mixta* was treated by Destruxin- 760and Destruxin A-724, respectively. After the Destruxin A-760 and Destruxin A-724 applied on *P. mixta*, the number of eggs was significantly decreased by 29.8 and 1.1% fold. Destruxin-724 caused higher malformations to sugar beet fly, *P. mixta* the toxin Destruxin-760 caused a reduced of the target insects. Under field conditions, sugar beet weight recorded 2497±93.19 and 2799±58.98 kg/ 4200 m^2 in plots treated with Destruxin 760 during seasons 2018 and 2019 respectively. Also the sugar beet weight increased after Destruxin -724 increased to 2415± 53.66 and 2625±68.91 Kg/fed. during seasons 2018 and 2019 , respectively as compared to 1680±65.43,1680±65.43 and 1120±85.09, 1120±85.09 kg/ feddan among the plots treated with water during the two successive season (2018 & 2019), respectively. use of *M. anisopliae* isolated toxin of Destruxin A-760, Destruxin - A-724 effect on the sugar beet fly, *P. mixta* larvae, pupae and adults in the laboratory. They also tested in the field and results obtained reduced the *P. mixta* numbers in the field.

Keywords: Sugar beet, Destruxin**,** *Pegomya mixta*, field application.

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INTRODUCTION

The Egyptian Sugar beet being a very important crop for producing sugar because the sugar beet contains a large amount of sugar reached to 20% (Bassyouny 1993and Abdel-Raheem 2000). Many pests causing a harmful injury in the sugar beet crop and cause crop destructions and yield loss (Bassyouny 1993).The harmful pests of Sugar beet fly, *Pegomyia mixta* Vill. which classify and belonging to (Diptera: Anthomyiidae), causing a damage and destructive to the planting of the sugar beet. The larvae of Sugar beet fly, *Pegomyia mixta* inter to the sugar beet leaves and burrow inside it causing a leaves blister and blotch by a thin trail in the leaves. At the end of the larval stage the pupae come out to the soil (Goodwin *et al.,* 2007, Zaki & Abdel-Raheem 2010 and Abdel-Moneim *et al.,* 2014). After the pupal stage ended the adults becomes after 2: 4 days many generations occur per year (Goodwin *et al.,* 2007). Sugar beet fly, *Pegomyia mixta* controlled by chemical harmful insecticides, so it is important to find another safe ways for

controlling this pest. Many fungi produce a toxins which killed the pests, *Metarhizium anisopliae* produce Destruxin which is the killer toxin causing insect death (Abdel-Raheem 2005, 2019, Saleh *et al.*, 2016, Abdel-Raheem *et al.*, 2016, 2019 & 2020, Abdel-Raheem 2017 & 2020a,b). Destruxin is a toxin of *Metarhizium anisopliae,* fungi. Mode of action of it is that causes a paralysis to the larvae, and adults then the speed death to infected insects. Destruxin causes infection to the insect immune system also it causes extinction to immune system. The Destruxin causes a decreasing in the leukemic cell production. When the Destruxin applied on the cotton leaf worm under laboratory conditions, the numbers of larvae and adults significantly decreased. They also, found that, the applications of Destruxin in the field, the infestations of cotton leaf worm, significantly decreased and there is a malformations observed among pupae and adults. Sabbour (2019) examine the Destruxin on aphids and the predators under laboratory condition, the aphids numbers is scientifically decreased and the predators is affected by a little. Two isolated Destruxin were evaluated on olive pests, the data obtained a number of reductions of the olive insects *Saissetia oleae* (Sabbour 2018). The white powder shape of chitosan (toxin) controlled the stored pests in the stores and laboratory. Our work aims to determine the effect of the toxin of Destruxin A-760 and Destruxin A-724 on *P. mixta* in the laboratory conditions and in the field conditions.

MATERIALS AND METHODS

Laboratory studies

The sugar beet fly, *P. mixta;* reared in the National Research Centre laboratory at the degree of (26± 2^{o}C and 60± 5 %R.H.). The sugar beet fly, *P. mixta* put in cages 70X 60X 80 cm per each. For laboratory tests, the 3rd larvae were used for all experiments.

Effect of Destruxin on *P. mixta*

Destruxin A-760 then Nano Destruxin A-624 were examined in the laboratory by six concentrations: they are 6 ppm, 5ppm, 4ppm, 3ppm, 2ppm, 1ppm. After the two Destruxins applications, mortality percentages was counted due to Abbott's formula, while the LC_{50} rate was counted throughout probit analysis (Abbott 1925). The experiment was carried out under laboratory conditions at 26±2°C and 60± 5 %R.H. The experiment repeated five times.

Field experiments

Experiments were carried out to study the efficacy of the tested 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. Sugar beet was planted at the first of September (2018) in an area of about 1200 m^2, divided into 12 plots of 100 m^{2}each. 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 5ppm concentration and 5Litre/plot. Treatments were performed in a randomized plot design at the sunset with a 5L sprayer. Three applications were made at 1WK interval at the commencement of the experiment, and then 20 samples of plants were randomly collected every week from each plot and transferred to laboratory for examination. Average number of each of the tested pest / sample / plot / treatment was calculated, 20, 50, 90- and 120-days post 1st application. After harvest, 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.

Statistical Analysis

We used the Duncan's various valuable tests due to (Finney 1971), also, the statistically analyzed LSD and *F*-test; by, using program of SPSS software.

RESULTS AND DISCUSSION

The LC_{50} of the Destruxin obtained 76 and 141 ppm after *P. mixta* treated with Destruxin A- 760, nano Destruxin A-724, respectively (Table 1). The effect of Destruxin on sugar

beet fly shown in (Table 2) the eggs laid per the females showed a decrease in numbers reached to 1±2.1and 27±9.7 eggs/ female but the untreated recorded 298±8.9 eggs /female. The egg hatching numbers, showed a significantly decrease to zero and four percent when sugar beet fly treated with Destruxin A-760 and Destruxin A- 724, respectively (Table 2). Also when the target insect treated with the corresponding toxins, adult malformed, the larval mortality, malformed pupae, significantly increased (Table 2).

Table 1. Laboratory evaluation the tested Destruxin A-724 and Destruxin A-760 on *P. mixta*

Toxins	LC_{50}	Slope	variance	Confidence limits at 95%
Destruxin A-724	141	0.1	1.4	
Destruxin A-760	76	1.1	1.1	60-139

Table 2. Effect of the Destruxin A-724 and Destruxin A-760 on biology of beet fly, *P. mixta*

Treatments	Eggs.NO. laid/female	Egg hatching %	Larval mortality %	Malformed larvae%	Malformed pupae%	f emerged adults%	Malformed adults %
Destruxin A-724	27±9.7[b]	4	61	66	77	3	78
Destruxin A-760	1±2.1[c]	0	91	97	98	-	-
Untreated	298±3.9[a]	100	0	0	0	100	0
F = value	31.9	1	4	4	20	21	23
Lsd5= %	11.2	2	5	4	11	11	11

After the harvest time the sugar beet roots collected and weighted, its weight recoded a significantly increased reached to 2497±93.19 and 2799±58.98 kg/ feddan in plots treated with Destruxin A-760 during seasons 2018 and 2019 respectively. Also the sugar beet weight increased after Destruxin -724 increased to 2415± 53.66 and 2625±68.91 Kg/4200 m ^{2} when Destruxin applied in seasons 2018 and 2019 , respectively as compared to 1680±65.43,1680±65.43 and 1120±85.09, 1120±85.09 kg/4200 m ^{2} found in the plots treated with water (control plots) this through season 2018 and 2019 respectively (Table 3).

Table 3. Sugar beet root weight obtained during field after applications of Destruxin A-724 Destruxin A-760

Toxins	Season 2018 Wt. of sugar beet (kg/ 4200m^2)	Season 2019 Wt. of sugar beet (kg/ feddan)
Destruxin A-724	2405± 53.66	2625±68.91
Destruxin A-760	2497±93.19	2799±58.98
Control	1680±65.43	1120±85.09

Economic feasibility study from sugar beet crop treating by using tested Destruxin applications.

The economic feasibility study requires the study and the evaluations by using the Destruxin A-724 and Destruxin A-760 in the 4200 m^2 productivity, quantity during irrigation water, the cost of producing tons of sugar beet, the yield of the pound invested from the farm, the pound invested from the transaction, the Egyptian exports and imports of sugar, which are as follows:

Productivity of 4200 m^2 Table (4) shows that the treatment of sugar beet plant using the mentioned materials contributed to increase the productivity of 4200 m^2 by about 80%, and about 89.14% when using Destruxin A-724 and Destruxin A-760 respectively, as shown by the total cultivated area of sugar beet in 2018 which amounted by 523,382 thousand feddans, and in the case of generalization of the total cultivated area of sugar beet, it will result in an increase of the amount produced by the crop by 8.689 sugar million tons, and about 9.681 million of sugar tons which give an approximately production reached to 1.231 million of sugar tons and 1.383 million of sugar tons are shown in (Table 5).

Irrigation Water: As can be seen from Table (4) that the treatment of sugar beet plant with

the above mentioned procedures which resulted in a decrease in the amount of irrigation water per ton of beet produced by about 44.4%, and about 47.1%, which entails the possibility of raising t34t5rhe efficiency of the irrigation water component compared to the current situation.

Table 4. Effect of Destruxin A-724 and Destruxin A-760 on Sugar Beet 4200m^2

Target pest	Average increase in production seasons%	Total area of sugar beet cultivated 4200m^2	Increase in production	Total production of sugar beet tons	Water quantity m ^{3} / ton	Cost of producing tons of sugar beet cultivated in pounds	the current situation	Pound yield per ton
Current situation	-	523382	10860921	10860921	192.758	482	600	1.245
Destruxin A-724	80.00	523382	8688737	19549658	107.088	268	600	2.239
Destruxin A-760	89.14	523382	9681425	20542346	101.913	255	600	2.353

Source: - Calculated from Table (3), Ministry of Agriculture and Land Reclamation - Statistical Bulletin 2017, Ministry of Irrigation Million Annual Bulletin 2017, Central Agency for Public Mobilization and Statistics

Production costs per ton: Table (4) shows that the cost per ton of sugar beet production decreased by 45.6% and about 47.1%, which lead to increasing the value of the pound invested return per ton produced by about 79.8%, and about 88.9% which compared to the current situation in the case of treatment Intentions in Destruxin A-724 and Destruxin A-760 respectively. As for the return of the pound invested for the feddan produced and the result of the treatment of the feddan in the above mentioned transactions, which lead to increasing by about 59.8%, and about 68% in compared to the current situation as shown in Table (5) in case the intentions are treated in Destruxin A-724 and Destruxin A- 760 respectively.

Egyptian Imports and Exports of Sugar: Increasing the production of sugar beet crop resulting from the treatment of plants using both Destruxin A-724 and Destruxin A-760 and generalization lead to increasing sugar production by about 1.231 million tons of sugar and about 1.383 sugar which contribute to reducing the amount of Egyptian imports of sugar to about one million tons, which contributes to saving about 562 million dollars, and leaving a surplus of produced sugar amounting to about 0.231 million of sugar tons, reached to 0.383 million of sugar tons, this actions can be directed by the state for export abroad achieve income of the state by about 120.81 million Dollars, and about $ 200.31 million and thus achieved a surplus in the commodity balance of sugar. The country is transformed from a sugar imported to a sugar exported as shown in Table (6).

Table 5. The added value of using Destruxin A-724 and Destruxin A-760

Target pest	Total cost per 4200m^2 in pounds	Feddan productivity	Total revenue	Investor Pound Return	The amount of increase in 4200m^2 per ton	The value of the increase in production	% Increase of the value of revenues
Current situation	10000	20.75	12450	1.245	-	-	-
Destruxin A-724	11268	37.35	22410	1.989	16.60	9960	44.44
Destruxin A-760	11255	39.25	23550	2.092	18.50	11100	47.13

Source: Calculated data through the Ministry of Agriculture and Land Reclamation - Annual Bulletin of Agricultural Statistics 2017

Figure 1 show that the sugar beet fly, *P. mixta* showed a significantly decrease in infestations when the two tested toxins applied in the field. These results meet with results recorded by (Sabbour 2014), extracted two types of Destruxin from the fungus *M. anisopliae* their results proved that these toxins reduce the infestations numbers of the *Hetiracris* Table 6. *littoralis* in the field and semi field. (Sabbour and Solieman 2014), proved that, three infested pests of sugar beet reduced in their numbers after using the fungi of *Beauveria brongniartii*. (Abdel-Raheem 2005, Sabbour and Abd El Rahman 2007) used the commercial terpenes with entomopathogenic.

Table 6. Evaluation the Destruxin A-724 and Destruxin A-760 on Egyptian Sugar Imports

Target pest	Total area of beet cultivated 4200m^2	Total production of beet tons	Total sugar produced	The amount of increase in sugar production is one million tons	The amount of imports of sugar million tons	The price of importing tons of sugar in dollars	The total value of imports is one million dollars	Export price of tons of sugar in dollars	Export revenue Million dollars
The current situation	523382	10860921	1551560	-	1	562	562	523	-
Destruxin A-724	523382	19549658	2782808	0.231	1	-	-	523	120.81
Destruxin A-760	523382	20542346	2934621	0.383	1	-	-	523	200.31

Source: Calculated from Table (3), United Nations Website, Central Agency for Public Mobilization and Statistics

fungi, their results obtained, that the three especially *Cassida vittata* in the laboratory pests of sugar beet decreased by these treatments in the field and reduced in numbers. Similarly, our results agree with (Sabbour 2015a) who found that, olive insects, *C. capitata*, and *P. oleae when* treated with nano materials they recoded a malformations among the larvae, pupae and adults. The same obtained by (Sabbour 2015b) found that *Phthorimaea operculella*, recoded a lesser in its numbers in the field after fungi treatments at especial doses. 4200 m^2. (Sabbour and Soleieman 2017), recorded that olive fruits produced by a higher weight when they apply microbial agents in the field. Also, the same results recorded by (Sabbour and Soleieman 2016), who apply many substance like Destruxin on the tomato leafminer, their found this pests reduced in numbers in the laboratory and decreased in numbers in the field. (Sabbour 2016) found the usage of Destruxin on the desert locust causing higher reductions in numbers of *Schistocerca gregaria* adults and nymphs.

Use of *M. anisopliae* isolated toxin of Destruxin A-760, Destruxin - A-724 effect on the sugar beet fly, *P. mixta* larvae, pupae and adults in the laboratory. They also tested in the filed conditions and results obtained reduced the *P. mixta* numbers in the field.

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