***Available online at* www.derpharmachemica.com**

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(18):132-138
(http://derpharmachemica.com/archive.html)

Isolates, Virulence of two Entomopathogenic Fungi as biological control agent on sugar beet fly, *Pegomyia mixta* in Egypt

Abdel-Raheem M. A., I. A. Ismail, N.A. Farag, R. S. Abdel-Rahman and H. H. Elbehery

Department of Pests and Plant Protection, National Research Centre, 33rd ElBohouth St. - Dokki, Giza, Egypt

ABSTRACT

This work was carried out of 200 samples from Abo-Ghalab- Kafr El-Shikh Governorate and Elnobaria - Elbehira Governorate only 30 samples (15 %) contained Entomopathogenic fungi, i.e., 18 isolates from Beauveria bassiana, 12 isolates from Metarhizium anisopliae. The fungi were propagated on Peptone media and rice grains. Peptone media gave the highest conidiospore production by B. bassiana and the rice media gave the highest conidiospore by M. anisopliae. Conidia were prepared in laboratory for bioassay at the concentrations of 2 x 10^2, 2 x 10^3 and 2 x 10^4 spores/ ml. (C_1, C_2 & C_3). Results obtained successful control against larvae of Galleria mellonella. Studies were carried out in the laboratory. The entomopathogenic fungi showed a high effect on Pegomyia mixta either in the laboratory and field experiments.

Keywords: Isolates, entomopathogenic fungi, *Pegomyia mixta*.

INTRODUCTION

Sugar beet, *Beta vulgaris* is one of the most important cultivations in Egypt and other countries. Sugar beet is cultivated for its roots (cone shaped white roots), which contain sucrose. Sugar beet plants are attacked by several insect pests and diseases, among these insects are the sugar beet fly, *Pegomyia mixta* which considered the most important insect pests in Egypt. This leaf miner lays eggs on the underside of the leave of small beets. Hatched larvae enter the leaf and mine out its inner portions. Several biological control techniques have been carried out in different countries Abdel-Moneim, *et al*., 2014 and Mohamed Abdel-Raheem, 2015. One of these methods was the use of entomopathogenic fungi *B. bassiana*, *M. anisopliae* and the bacteria *Bacillus thuringiensis* (Dipel 2x). In 2004, Ibrahim, *et al.* reported that Dipel 2x, eliminated 15.82% of *P. mixta*, while *B. bassiana* suppressed 35.53 of the population Abdel-Raheem, 2005 and Zaki and Abdel-Raheem, 2010. Veen and Ferron, 1966, Salem, *et al.*, 2015, Abdel-Raheem,et al., 2015, 2016, Ismail, et al., 2015, 2016, Sabbour and Abdel-Raheem, 2016 and Saleh, *et al.*, 2016 isolated some entomopathogenic fungi and used it to control insect pests in sugar beet and other crops in Egypt.

MATERIALS AND METHODS

Entomopathogenic Fungi:
Soil samples were collected from Kafr El-Shikh and Elbehira Governorates from the fruit farms for trapping entomopathogenic fungi following the technique created by Zimmermann (1986 & 1998) using larvae of the grater wax moth, *Galleria mellonella* L. as a susceptible insect host. Dead larvae were collected from soil samples, washed with sterilized distilled water and kept in Petri-dishes on moistened filter paper at 24 ±2 ºC and 85± 5 % RH. .

Fungi cultures:
The tested entomopathogenic Fungi *Metarhizium anisopliae* (Metchinkoff) Soroken and *Beauveria bassiana* (Balsamo) Vuillemin isolated from soils, and grown on peptone media (10g Peptone, 40g Dextrose, 2g Yeast extract, 15g Agar and 500 ml. Chloramphenicol and completed to one liter by distilled water). The media was autoclaved at

120 ◦c for 20 minutes, and poured in Petri-dishes (10 cm diameter x 1.5 cm) then inoculated with the two entomopathogenic fungi and kept at 25 ±2◦C and 85 ±5 R.H. The fungal isolates were re-cultured every 14-30 days and kept at 4 ◦C.

To obtain a huge numbers of conidia, both *B. bassiana* and *M. anisopliae* isolates were propagated on wetted rice. Two Kilos gram wetted rice was washed in boiled water for 10 min. and put in thermal bags. These bags were autoclaved at 120ºC for 20 min., then infected by both isolates and incubated at 26 ± 1 ºC for 15 days. The Conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added.

Preparing of the concentrations:
Conidia of fungal isolates harvested by rising with sterilizing water 0.5% Tween 80 from 14 days old culture rice media. The suspensions were filtered through cheese cloth to reduce mycelium clumping. Conidia were counted in the suspension by using a haemocytometer (Hirscmann 0.1 mm x 0.0025 mm^2). The suspension was put in plastic bottles (2 liter). To restore the virulence of the isolates it was passed through their natural host, wax moth larvae *Galleria mellonella.* Three concentrations were prepared, (C_1) $2x10^2$, (C_2) $2x10^3$ and (C_3) $2x10^4$ Spores /ml in both entomopathogenic fungi.

Bioassay procedure:
1- Effect of *B. bassiana* and *M. anisopliae* isolates on eggs of *Pegomyia mixta*:
The eggs of *Pegomyia mixta* were treated with the following concentrations ($2x10^2$, $2x10^3$ and $2x10^4$ spores / ml.) each concentrations includes three replicates. Each replicate contained five eggs.
2- Effect of *B. bassiana* and *M. anisopliae* isolates on the larvae of *Pegomyia mixta*:
The larvae of *Pegomyia mixta* were put in Petri dishes (12 cm diameter) placed a wetted filter paper and dipped in the previous concentrations.
3- Effect of *B. bassiana* and *M. anisopliae* isolates on the pupae of *Pegomyia mixta*:
The pupae of *Pegomyia mixta* were put in Petri dishes (12 cm diameter) placed a wetted filter paper and dipped in the previous concentrations.

Field experiments:
Experiments were carried out to study the Virulence of the tested fungi against the target insect pests, during the two successive seasons 2014&2015 starting from the first of March 2014 to evaluate the virulence of the tested fungi against *Pegomyia mixta* under field conditions. Four random plots of sugar-beet were selected; each for *B. bassiana, M. anisopliae* and the other for control) to carry out the field experiment.

B. bassiana and *M. anisopliae* were applied, each as a single treatment at the rate of $2x10^4$ spores / ml.. Treatments were performed at the morning early with a ten liter sprayer. Percentage of mortality /plot was calculated after 7, 14, and 21 days of the application. Each treatment was replicated three times.

The reduction percentages in the population density of *Pegomyia mixta* in relation to the pre-treatment count were calculated according to Henderson and Tilton formula, (1955) as follows:

$$\text{Reduction percentages} = 1 - \frac{Ta\ Cb}{Tb\ Ca} \times 100$$

Where: Tb and Ta are pre- and after-treatment counts, respectively.
Cb and Ca are untreated checks before and after treatment.
Data of the percentages reduction were subjected to simple analysis of variance.

RESULTS

The data in table (1) obtained the entomopathogenic Fungi *Metarhizium anisopliae* (Metchinkoff) Soroken and *Beauveria bassiana* (Balsamo) Vuillemin isolated from soils. The fungi positive of *B. bassiana* was 10% from Abo-Ghalab and 8% from Elbehira. The fungi positive of *M. anisopliae* was 8% from Abo-Ghalab and 4% from Elbehira .

Table (1): Numbers of positive entomopathogenic fungi recovery by trapping with larvae of *G. mellonella* from soil samples collected from Abo-Ghalab and Elnobaria in Egypt and their rates of incidence (%)

Plant	Number of samples	Fungi positive samples and incidence (%)	
		B. bassiana	*M. anisopliae*
Kafr El-shikh	100	10 (10 %)	8 (8 %)
Elbehira	100	8 (8 %)	4 (4 %)
		18 (9 %)	12 (6 %)
Total	200	(9 %)	(6 %)
		15 %	

Table (2) showed that the percent mortalities are occurred in the third day of *Galleria mellonella* larvae treated with *Beauveria bassiana* isolate from Kafr El-Shikh. The percent of mortalities are increased gradually and reached to the maximum (100 %) mortality when treated with the concentration (C_3) after 8th day from treatment and reached to 100 % mortality after 9th day when treated with the concentration (C_3) from *M. anisopliae*). The percent of mortalities ranged between 92.0 to 100 and 60.5 to 80% with *B. bassiana* and *M. anisopliae*, respectively, in the 8th day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation at 8th day were 92, 97 and 100 %, respectively. The corresponding results with *M. anisopliae* isolation were 60, 78 and 80%, respectively.

Table (2): % Mortality *Galleria mellonella* larvae treated with *Beauveria bassiana,* and *Metarhizium anisopliae* isolates from Kafr El-Shikh

Days after treatment	% of Mortality						
	control	*B. bassiana* KB1			*M. anisopliae* KM1		
		*C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	10.5	13.5	15.5	8.5	9.5	12.5
4th	0.0	15.5	17.0	18.5	10.0	14.5	16.5
5th	0.0	16.0	17.5	20.0	13.0	16.5	18.0
6th	0.0	40.0	43.0	60.0	40.0	41.0	44.5
7th	0.0	70.0	75.5	85.0	55.5	70.0	73.0
8th	0.0	92.0	97.0	100	60.5	78.0	80.0
9th	0.0	100	100	100	72.0	82.0	100

**C_1 = 2x10^2^, C_2 =2x10^3^ spores/ ml. C3 = 2x10^4^ spores/ ml.*

Table (3) showed that the percent mortalities are of *Galleria mellonella* larvae treated with *Beauveria bassiana* isolate from Elbehira were reached to 100% mortality when treated with the concentration (C_3) after 7th day from treatment and reached to 100 % mortality after 9th day when treated with the concentration (C_2& C_3) from *M. anisopliae.*

The percent of mortalities ranged between 95 & 100 and 75 & 85 % with *B. bassiana* and *M. anisopliae*, respectively, in the 7th day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation at 7th day were 79, 95 and 100 %, respectively. The corresponding results with *M. anisopliae* isolation were 60, 75 and 85%, respectively.

Table (3): % Mortality *Galleria mellonella* larvae treated with *Beauveria bassiana,* and *Metarhizium anisopliae* isolates from Elbehira

Days after treatment	% of Mortality						
	control	*B. bassiana* EB1			*M. anisopliae* EM1		
		*C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	15	20	25	12	15	15
4th	0.0	25	33	40	15	16	20
5th	0.0	35	49	60	25	30	45
6th	0.0	55	65	75	40.0	45	50
7th	0.0	79	95	100	60	75	85
8th	0.0	100	100	100	70	80	94
9th	0.0	100	100	100	80	100	100

C_1 = 2x10^2^, C_2 =2x10^3^ spores/ ml. C3 = 2x10^4^ spores/ ml.

Table (4) showed that the percent mortality of *Pegomyia mixta* eggs treated with *Beauveria bassiana* isolates from Kafr El-Shikh were reached to 100% mortality when treated with the concentration (C_3) after 6th day from treatment and reached to 73 % mortality after 6th day when treated with the concentration (C_3) from *M. anisopliae.*

The percent of mortalities ranged between 60 to 100 and 45 to73 % with *B. bassiana* and *M. anisopliae*, respectively, in the 6th day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation at 6th day were 60, 75 and 100 %, respectively. The corresponding results with *M. anisopliae* isolation were 45, 60 and 73%, respectively.

Table (4): % Mortality *Pegomyia mixta* eggs treated with *Beauveria bassiana,* and *Metarhizium anisopliae* isolates

Days after treatment	% of Mortality						
	control	*B. bassiana* KB1			*M. anisopliae* KM1		
		C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4th	0.0	10	13	20	8	11	14
5th	0.0	45	55	60	25	33	50
6th	0.0	60	75	100	45	60	73

**C_1 = 2x10^2^, C_2 =2x10^3^ spores/ ml. C3 = 2x10 ^4^ spores/ ml.*

Table (5) showed that the percent mortality of *Pegomyia mixta* larvae treated with *Beauveria bassiana* from Kafr El-Shikh were reached to 100% mortality when treated with the concentration (C_3) after 10th day from treatment and reached to 100 % mortality after 11th day when treated with the concentration (C_3) from *M. anisopliae.*

The percent of mortalities ranged between 90 to 100 and 66 to75 % with *B. bassiana* and *M. anisopliae*, respectively, in the 10th day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation at 10th day were 90, 92 and 100 %, respectively. The corresponding results with *M. anisopliae* isolation were 66, 65 and 75%, respectively.

Table (5): % Mortality *Pegomyia mixta* larvae treated with *Beauveria bassiana,* and *Metarhizium anisopliae* isolates

Days after treatment	% of Mortality						
	control	*B. bassiana* KB1			*M. anisopliae* KM1		
		*C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4th	0.0	13	15	18	10	14	16
5th	0.0	32	33	44	17	32	44
6th	0.0	40	43	60	40	41	44
7th	0.0	70	75	85	55	55	66
8th	0.0	73	77	87	55	60	77
9th	0.0	80	83	97	60	60	77
10th	0.0	90	92	100	66	65	75
11th	0.0	100	100	100	73	80	100

**C_1 = $2x10^2$, C_2 =$2x10^3$ spores/ ml. C3 = 2x10 ^{4} spores/ ml.*

Table (6) showed that the percent mortality of *Pegomyia mixta* pupae treated with *Beauveria bassiana* from Kafr El-Shikh were reached to 100% mortality when treated with the concentration (C_3) after 12th day from treatment and reached to 100 % mortality after 14th day when treated with the concentration (C_3) from *M. anisopliae.*

The percent of mortalities ranged between 92 to 100 and 60 to80 % with *B. bassiana* and *M. anisopliae*, respectively, in the 12th day after treatment. This means that *B. bassiana* isolation is more effective than *M. anisopliae*. The percent of mortalities with all concentrations (C_1, C_2 and C_3) of *B. bassiana* isolation at 12th day were 92, 97 and 100 %, respectively. The corresponding results with *M. anisopliae* isolation were 60, 78 and 80%, respectively.

Table (6): % Mortality *Pegomyia mixta* pupae treated with *Beauveria bassiana,* and *Metarhizium anisopliae* isolates

Days after treatment	control	% of Mortality					
		B. bassiana KB1			*M. anisopliae* KM1		
		*C_1	C_2	C_3	C_1	C_2	C_3
2nd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3rd	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4th	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5th	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6th	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7th	0.0	10	13	15	8	11	15
8th	0.0	18	19	27	11	18	33
9th	0.0	20	26	45	13	23	35
10th	0.0	40	43	60	40	45	44
11th	0.0	70	75	85	55	70	73
12th	0.0	92	97	100	60	78	80
13th	0.0	100	100	100	72	82	96
14th	0.0	100	100	100	85	86	100
15th	0.0	100	100	100	100	100	100

C_1 = $2x10^2$, C_2 = $2x10^3$ spores/ ml. C3 = $2x10^4$ spores/ ml.

Field experiments:

An area of about 2100 m^2 was chosen in Abo-Ghalab region, Kafr El-Shikh Governorate, cultivated with sugar beet plants in mid-September 2014. Infestation with *P. mixta* started to appear during the second week of January 2015. In mid- February, the first application was carried out using two entomopathogenic fungi *Beauveria bassiana,* and *Metarhizium anisopliae*

Table (7): Field application with *Beauveria bassiana,* and *Metarhizium anisopliae* on *Pegomyia mixta* at Kafr El-Shikh Governorate season (2014-2015)

Treatment	Virulence of *B. bassiana,* and *M. anisopliae*			
	No of infested / 10 plants	No of infested leaves	No of infested / egg patch / plant	No of infested larvae / plant
6 March				
Control	7 (70%)	12	10	20
B. bassiana	7 (70%)	11	13	22
M. anisopliae	8 (80%)	12	12	21
13 March				
Control	7 (70%)	13	11	21
B. bassiana	1 (10%)	4	5	4
M. anisopliae	2 (20%)	6	6	15
20 March				
Control	6 (60%)	12	10	20
B. bassiana	0.0 (0 %)	0.0	0.0	0.0
M. anisopliae	1 (10 %)	2	3	0.0

The number of infested / 10 plants was 70 % before the first spray by *B. bassiana* and reached to 10 % after seven days from treatment and reached to zero% infested after 14 day from first treatment. Also, The number of infested leaves 11 before the first spray by *B. bassiana* and reached to 4 after seven days from treatment and reached to zero infested after 14 day from first treatment.

The number of infested egg patch / plant 13 before the first spray by *B. bassiana* and reached to 5 after seven days from treatment and reached to zero infested after 14 day from first treatment.

The number of infested larvae / plant 22 before the first spray by *B. bassiana* and reached to 4 after seven days from treatment and reached to zero infested after 14 day from first treatment.

The number of infested / 10 plants was 80 % before the first spray by *Metarhizium anisopliae* and reached to 20 % after seven days from treatment and reached to 10% infested after 14 day from first treatment. Also, the number of infested leaves 12 before the first spray by *B. bassiana* and reached to 6 after seven days from treatment and reached to 2% infested after 14 day from first treatment.

The number of infested egg patch / plant 12 before the first spray by *B. bassiana* and reached to 6 after seven days from treatment and reached to 3% infested after 14 day from first treatment.

The number of infested larvae / plant 21 before the first spray by *B. bassiana* and reached to 15 after seven days from treatment and reached to zero infested after 14 day from first treatment.

Table (8): Field application with *Beauveria bassiana,* and *Metarhizium anisopliae* on *Pegomyia mixta* at Kafr El-shikh Governorate season (2015-2016)

Treatment	Virulence of *B. bassiana,* and *M. anisopliae*			
	No of infested / 10 plants	No of infested leaves	No of infested / egg patch / plant	No of infested larvae / plant
5 March				
Control	6 (60%)	14	12	25
B. bassiana	6 (60%)	13	12	25
M. anisopliae	7 (70%)	13	12	23
12 March				
Control	6(60%)	14	13	25
B. bassiana	2 (20%)	3	4	12
M. anisopliae	3 (30%)	6	6	14
19 March				
Control	7 (70%)	13	13	24
B. bassiana	0.0 (0 %)	0.0	0.0	0.0
M. anisopliae	0.0 (0 %)	0.0	0.0	0.0

The number of infested / 10 plants 60 % before the first spray by *B. bassiana* and reached to 20 % after seven days from treatment and reached to zero% infested after 14 day from first treatment. Also, The number of infested leaves 13 before the first spray by *B. bassiana* and reached to 3 after seven days from treatment and reached to zero infested after 14 day from first treatment.

The number of infested egg patch / plant 12 before the first spray by *B. bassiana* and reached to 4 after seven days from treatment and reached to zero infested after 14 day from first treatment.

The number of infested larvae / plant 25 before the first spray by *B. bassiana* and reached to 12 after seven days from treatment and reached to zero infested after 14 day from first treatment.

The number of infested / 10 plants 70 % before the first spray by *Metarhizium anisopliae* and reached to 30 % after seven days from treatment and reached to Zero% infested after 14 day from first treatment. Also, the number of infested leaves 13 before the first spray by *Metarhizium anisopliae* and reached to 6 after seven days from treatment and reached to Zero% infested after 14 day from first treatment.

The number of infested egg patch / plant 12 before the first spray by *Metarhizium anisopliae* and reached to 6 after seven days from treatment and reached to Zero% infested after 14 day from first treatment.

The number of infested larvae / plant 23 before the first spray by *Metarhizium anisopliae* and reached to 14 after seven days from treatment and reached to zero infested after 14 day from first treatment.

Finally, these data clear that the entomopathogenic fungi *B. bassiana* and *Metarhizium anisopliae* can be used as a promising agent in pest control and integrated pest management programs instead of conventional pesticides to reduce the environmental pollution especially when the pests were under the economic threshold.

DISCUSSION

This study carried out some isolates from soil, *B. bassiana* and *M. anisopliae* and used it as agent control and evaluate the virulence, Abdelraheem, 2005 isolated *B. bassiana* and *M. anisopliae* from *Cassida vittata* and *Scrobipalpa ocellatella* and used to control the insects, Salem, et al., 2015 took Soil samples from different governorates i.e., Elbehaira (119 samples), Kafr El-Shaeikh (103 samples) and Aswan (97 samples) were investigated. Only 10 samples (3.134 %) contained entomopathogenic fungi, i.e., 7 isolates contain *Beauveria Bassiana* and 3 isolates contain *Metarhizium Anisopliae*. and Saleh, et al., 2016 studied

Abundance of entomopathogenic fungi (EPF) in soil of fruit orchards located at Alexandria desert road, Egypt, was studied through biweekly soil samples collected from January to June 2015. Three entomopathogenic fungi could be isolated and identified namely: *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii. B. bassiana* was the most abundant fungus forming 25% of total collected samples followed by *M. anisopliae* (17.76%) and *V. lecanii* (14.49%). All fungi occurred heavily during January to March and disappeared gradually during April and May.

The entomopathogenic fungi, *B. bassiana* was more virulence than *M. anisopliae* according Abdel-Raheem, 2005 and Zaki and Abdel-Raheem, 2010.

This result compatible with Maniania (1991) who found that both of *B. bassiana* and *V. lecanii* caused mortalities of up to 97 and 100% in *Chilo partellus*, respectively. Zaki (1998) reported that *B. bassiana* as an entomopathogenic fungi showed high effects on the aphid *Aphis craccivora* and the white fly *B. tabaci* infesting cucumber. Abdel-Baky *et al.* (2005) mentioned that entomopathogenic fungi caused good mortality to whitefly.

Acknowledgment
This research has been financed by National Research Centre, Giza, Egypt. Project No P 100707.

REFERENCES

[1] Abdel-Moneim, A.S.H. Abdel-Raheem , M. A. and Elkholy , M. Y. *Archives of Phytopathology and Plant Protection*, **2014** Vol. 47, No. 13, 1557–1562.
[2] Mohamed Abdel-Raheem ISBN: (978-3-659-81638-3), 2015 76Pp.
[3] Ibrahim I. Mesbah , Fayez A. Abou- Atia, Shawky M. Metwally , Ahmed M. Bassyouni and Gamal A. *Egyptian Journal of Biological Pest Control*, **2004**, 14 (1), **2004**, 195 -199
[4] Abdel-Raheem, M. A. Ph.D. Faculty of Agriculture, Cairo University, Cairo.**2005**, 86Pp.
[5] Zaki, F.N. and Abdel-Raheem, M.A. *Archives of phytopathology and plant protection*. **2010**, Vol. 43, No. 18, 10 December **2010**, 1819-1828.
[6] Veen KH, Ferron P. *J. Invertebr Pathol*. . **1966**, 8, 268-269.
[7] Salem, S.A.; Abdel-Raheem, M.A.; Abdel-Salam,A.M.A. and Farage N.A. *Swift journals of Agricultural Research.* **2015**, Vol 1(2) pp. 009-014 June, 2015
[8] Abdel-Raheem, M.A.; I.A. Ismail; R.S. Abdel-Rahman; N.A. Farag and I. E., Abdel-Rhman. *Journal of Entomology and Zoology Studies*. **2015**; 3(5)329:333
[9] Abdel-Raheem, M. A.; Naglaa F. Reyad ;Abdel-Rahman, I. E.and Al-Shuraym, Laila. A. *International Journal of ChemTech Research*, **2016**, Vol.9, No.8, Pp. 479-485.
[10] Abdel-Raheem, M. A.; Naglaa F. Reyad ; Al-Shuraym, Laila. A. and Abdel-Rahman, I. E. *Der Pharmacia Lettre* **2016**, (in press)
[11] Ismail, I.A.; R.S. Abdel-Rahaman and M.A. Abdel-Raheem. *International Journal of Chem. Tech Research*. **2015**, Vol.8, No.9, pp 187-195.
[12] Ismail, I.A. and R.S. Abdel-Rahman and M.A. Abdel-Raheem . *International Journal of ChemTech Research*, **2016**, 9 (4):35-42.
[13] Sabbour M.M and M.A. Abdel-Raheem. *Der Pharma Chemica*, **2016**, 8(4):133-136.
[14] Saleh, M.M.E., M.A. Abdel-Raheem., I.M. Ebadah and Huda H. Elbehery. *Egyptian Journal of Biological Pest Control*, 2016,26(2), **2016**, 203-207.
[15] Zimmermann, G. *Journal Applied Entomology*,**1986**, 102(2) 213-215.
[16] Zimmermann G. *Insect pathogens and insect parasitic nematodes*. IOBC Bull, **1998**, 21(4), 289.
[17] Henderson, C. F .and E. W. Tilton *J .Econ Entomol*., **1955**, 48: 157 – 161 .
[18] Maniania, N. K. (*J. Appl. Entomol.* **1991**, *112: 53-58).*
[19] Zaki, F. N. *J. of Applied Entomol.*, **1998**, *122 : (7) 397-399).*
[20] Abdel-Baky, N. F.; EL-Fadaly, H.A., El-Nagar M. E., Arafat, N. S. and Abd-Allah, R. R. H. *J. Agrie. Sci., MansouraUni.* **2005**, *30: 1153-1167).*