J. Plant Prot. and Path., Mansoura Univ., Vol.6 (5): 727 - 738, 2015

INSECTICIDAL POTENCY OF NATIVE ENTOMOPATHOGENIC FUNGI ISOLATES AGAINST THE *Galleria mellonella* (LEPIDOPTERA: PYRALIDAE) AND *Spodoptera littoralis* (LEPIDOPTERA: NOCTUIDAE) *Larvae.*

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ABSTRACT

The entomopathogenic fungi (EPF) have received considerable attention for their potential use in biological control of insect pests. In this study, twenty four native strains were isolated from Alexandria governorate. This isolates were identified as 21 *Beauveria bassiana* (Ascomycota: Hypocreales) isolates and three isolates of *Metarhizium anisopliae* (Ascomycota: Hypocreales). Isolates were preliminary evaluated at 1x108 conidia ml-1 under laboratory conditions against 3rd instar larvae of *Spodoptera littoralis* and 4th instar of G. mellonella larvae. In addition, concentration-mortality relationship was conducted for selected three native *B. bassiana* isolates compared with two exotic isolates *B. bassiana* (Bio-power) and *M. anisopliae* (Bio-magic). The result revealed that the larvae of *G. mellonella* were more sensitive than *Spodoptera littoralis* to all EPF isolates with LT_{50} range (6.28–11.21days) and (7.81–13.28 days) respectively. The results indicated a significance difference (α = 0.05) for 13 native EPF isolates in their speed of kill (LT50) toward G. mellonella than S. littoralis. In addition, the concentration–mortality relationship assay showed that larvae mortality increase in a linear relationship with conidia concentration and the Bb-Mo12 a native isolate causing higher mortality percentage in both tested larvae, while Bio-power caused the lower mortality percentage in both larvae at higher tested concentration. The result show that Bb-Mo12 isolate have a lower LC_{50} $1.3X10^6$ and $1.1X10^7$ conidia ml-1 toward G. mellonella and S. littoralis larvae respectively. from these results, we could conclude that there are a variation between native EPF isolates in their virulence toward insect pest and some isolates have a promising potential for use as biocontrol agents in the field of insect control.

Keyword: Native, virulence, *Galleria mellonella*, *Spodoptera littoralis*, entomopathogenic fungi, biological control.

INTRODUCTION

The cotton leafworm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) is one of the important widespread pests that cause great damage to cotton plants as well as other field and vegetable crops in Egypt and subtropical region in the world (Willcocks and Bahgat, 1937; Moussa, et.al., 1960; Bishara, 1954). It have a wide range of host plant subject to 27 plant species belonging to 16 families around the year (Salama, et al., 1971; Anderson et al., 2001). It cause a significance damage and loss in the yield of several economically crops (Carter, 1984)

The products of beehives such as wax have a wide usage in pharmaceutical industry, health care, dentistry and cosmetics in addition to their nutritional value, which limit the usage of pesticide in the control of

insect pests inside the beehives. The greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae) considered a serious pest of beehives which cause a damage through feeding upon pollen and tunnelling through the combs (Jackman and Drees 1998; Chandel et al. 2003). Wax moth damage only during their larval stage. The moth is widely distributed throughout the world, causing serious problems in temperate, tropical and subtropical beekeeping regions, where the warm temperature favour the rapid development of the moth (Spangler 1989).

The rapid increase in human population with changes in dietary habits towards high quality food required is thought to cause more than double demands for crop production. However, the climate changes will add to the crisis (Tubiello et al., 2007). as agricultural production intensified over the past few decades, producers became more dependent on agrochemicals and the chemical pesticides become the main agents used in crop protection,

The conventional chemical pesticides have although enhanced the food production, but have also adversely affected the environment and non target organisms. In addition Chemical pesticides are continuously accumulating in the environment, harming the ecosystem, causing pollution,(Gerhardson 2002).

There are increasing pressure to move towards more environmentally friendly biological control systems (Hall and Papierok, 1982; Moore and Prior, 1993). A promising strategy with good potential to control insect pests in addition to minimize adverse effects of chemical insecticides is the use of entomopathogenic microbial agents such as fungi. Mycoinsecticides are safer than chemical insecticides in that they are selective, biodegradable, can be incorporated into IPM programs, and by remaining in the environment as successive generations they may give extended control (Moore and Prior, 1993). There are more than 800 species of fungi belong to more than 90 genera that are pathogenic to insects (Thackar, 2002). The Metarhizium and Beauveria being the best known genera as they have a wide geographical distribution and host range (Hall and Papierok, 1982; McCoy et al., 1988). Fungal biological control agents have demonstrated efficacy against a wide range of insect pests including Spodoptera species and *Galleria mellonella* (Purwar and Sachan, 2005; Lin et al., 2007; Shairra and Gehan 2014; Gabarty *et al,* 2014 and Hussein, et al., 2012).

The objectives of this study were to assess the insecticidal activity of native isolates of entomopathogenic fungi against 3rd instar larvae of *S. littoralis* and 4th istar larvae of *G. mellonella* in addition to comparative study of selected native and exotic isolates against the same tested larvae.

MATERIALS AND METHODS

Rearing of tested insects

A susceptible Egyptian cotton leafworm, *S. littoralis*, strain was obtained from the biological control laboratory, department of economic entomology faculty of agriculture, Alexandria university. The colony was reared under laboratory conditions on castor oil leaves at 26°C±2 , 65±5 RH (El-Defrawi *et al.,* 1964).

The greater wax moth, *G. melonella* (L.) larval were obtained from the infested hives and reared in the laboratory at temperature of 28±2°C using the following artificial diet composition: wheat bran, 100g; wheat flour, 100 g; cornmeal, 200 g; milk powder 100g; dried yeast, 50g; glycerin, 175 ml; and honey, 175 ml. (PDBC, 2007)

Entomopathogenic fungi isolates.

Twenty-four native isolates of EPF used in this study. Three of them belong to *Metarhizium anisopliae* and twenty-one isolates are *B. bassiana,* all isolates were isolated by the insect-baiting technique (Zimmermann, 1986) from soil samples collected during 2012-2013 from Alexandria governorate in Egypt. The identification of fungal isolates based on mycelium growth on the insect and after isolation on media by spore morphology and colour (Samson et al. 1988 and Humber 1997)

From the fungal species, local district name the soil sample were collected, and from positive sample sequence number. The name code for isolates of EPF was designated, according to Montaza district (Mo), Borg elarab district (Bo), Amria district (Am) and eastern district (Ea). Abbreviate for fungal species indication *B. bassiana* (Bb) *M. anisopliae* (Ma), and sample number (1,2,3); thus isolates were labeled Bb-Mo1, Bb-Mo2, Bb-Bo3, Ma-Am1..etc.

Two entomopathogenic fungi isolates used as exotic isolates for comparative study, *M. anisopliae* (Bio-magic) and *B. bassiana* (Bio-power) available commercially from T. Stanes Company limited, India.

Preparation of fungal inoculum.

The isolates was grown on potato dextrose agar medium supplemented with 1% yeast extract (PDAY) in petri dishes (90 mm) in the dark at 28±1 °C for 14 days. The conidia for each fungal isolates was harvested by scraping from the surface of plates with a sterile scalpel, and suspended in a sterile aqueous solution of 0.05 % Tween 80. The suspension was homogenized by shaken vigorously using a vortex mixer and filtered through sterile muslin to remove mycelia and debris. The conidia concentrations was quantified microscopically using a hemocytometer. The dilutions were made with the 0.05% (v/v) Tween 80 solution. To prevent germination of conidia before use, the suspensions was placed overnight in a refrigerator at 4 °C. The fungal isolates were used in assay after not more than two subcultures on PDAY to avoid possible loss of virulence associated with continuous culturing (Hajek et al. 1990)

Virulence assays.

The 3rd instar larvae of *S. littoralis* and the 4th instar larvae of *G. mellonella* were used in the experiment. A batch of ten larvae had been used for each fungal isolates for each larvae, three replicates was maintained for each fungal isolate. For inoculation, each group of larvae were sprayed by 5 ml of conidial suspension at 10^8 conidia ml^{-1} in the preliminary bioassay for native isolates and the concentrations 10^6, 10^7, 10^8 and 10^9 conidia ml^{-1} for concentration-mortality relationship study, using a hand atomizer for each fungal isolates. The control larvae received a spray of 0.05% tween 80 solution in distilled water, after air-drying, each group was placed in a

sterilized moist chamber consisting of a petri dish lined with wet filter paper. The experiments conducted at laboratory condition 27 ± 2°C and 75±10 relative humidity. Mortality of larvae was assessed daily up to ten days after inoculation.

Statistical analysis

Time- mortality and concentration-mortality data was performed using probit analysis and calculate regression lines slope and LT_{50} (median lethal time) or LC_{50} (median lethal concentration) values were considered significantly different if the 95% confidence limits did not overlap. To assess the differences in insect mortality percentage among *S. littoralis* and *G. mellonella* larvae for each tested native and exotic EPF isolates, the percent mortality were corrected according to Abbott's formula (Abbott, 1925), and subjected to arcsine square root transformation to increase the homogeneity of variance and normality. Then the data subjected to statistical analysis of variance (ANOVA) using SAS software version 9.0 (SAS Institute, Cary, NC 2004). Means was separated using LSD test and differences at $P \leq 0.05$ were considered significant.

To assess the combined effect of Host larvae species, EPF isolates and EPF concentration on the Corrected cumulative mortality percentage, a fully randomised factorial design was used. The main effect of each factor and their interaction was analysed using the general linear model (GLM) procedure available within the SAS statistical software 9.0 (SAS Institute Inc., 2004), the data used for analysis was arcsine transformed.

RESULTS

From 1100 soil samples collected from different district in Alexandria city, Egypt about 2.18% of the sample were positive for the presence of entomopathogenic fungi, 21 isolates of *B. bassiana* and 3 isolates of *M. anisopliae* were isolated on potato dextrose agar + yeast extract (PDAY).

Data present in Table (1) indicate that, both tested insect larvae *S. littoralis* and *G. mellonella* were susceptible to all native isolates of entomopathogenic fungi. There are 13 isolates have a significance difference ($P < 0.05$) in their virulence toward the tested larvae with high speed of kill (low LT_{50}) against *G. mellonella* in comparing with *S. littoralis* larvae these isolates are Bb-Mo1, Bb-Mo21, Bb-Ea6, Bb-Ea7, Bb-Ea11, Bb-Am4, Bb-Am8, Bb-Mo17, Bb-Am20, Ma-Am1, Bb-

For the native M. anisopliae isolates we can observe that Ma-Am1 isolate have a significance low LT_{50} ($P < 0.05$) in compare with Ma-Bo2 and Ma-Bo3 against G. mellonella larvae while no significance difference observed in their LT_{50} value toward *S. littoralis* larvae.

Table (1): LT_{50} and their 95% confidence limit, slope± SE and X^2 for 24 native EPF isolates on two lepidopteron larvae *S. littoralis* and *G. mellonella*.

Fungi isolate	*G. mellonella*				*S. littoralis*			
	LT_{50} (day)	Confidence limit (95%)	Slope ± SE	X^2	LT_{50} (day)	Confidence limit (95%)	Slope ± SE	X^2
Bb-Mo1	7.67	6.98 - 8.62	4.38±0.64	1.24	11.24	9.57 -16.13	4.02 ±0.9	0.39
Bb-Mo2	11.21	9.19 – 19.18	2.93±0.78	0.34	12.74	10.41 - 21.77	3.91±0.98	0.51
Bb-Mo12	6.60	6.06 – 7.16	5.37 ±0.78	3.1	7.81	7.141 - 8.749	4.73±0.78	0.35
Bb-Mo13	10.07	8.72 – 13.54	3.67±0.81	1.49	11.19	9.50 - 16.24	3.85±0.88	0.33
Bb-Mo15	8.09	7.38 – 9.15	4.68±0.79	0.76	9.75	8.7 – 13.02	4.62±0.89	0.13
Bb-Mo17	6.29	5.8 – 6.86	5.47±0.74	1.73	10.95	9.14 – 17.14	3.18±0.79	0.41
Bb-Mo19	9.01	8.22 – 10.39	5.24±0.9	0.11	10.71	9.11 – 15.38	3.57±0.82	0.29
Bb-Mo٢١	7.07	6.43 – 7.88	4.23±0.61	0.51	12.78	10.14 –25.35	3.09±0.84	0.14
Bb-Ea6	7.94	7.28 – 8.87	4.96±0.8	0.51	12.25	10.15 –19.63	3.95±0.96	0.94
Bb-Ea7	6.28	5.77 – 6.81	5.84±0.92	2.34	11.48	9.43 – 19.01	3.03±0.79	0.73
Bb-Ea11	7.21	6.62 – 7.92	4.97±0.77	0.19	12.08	9.88 - 20.44	3.41±0.86	0.44
Bb-Ea14	10.42	8.88 -14.86	3.43±0.8	0.29	10.65	9.12 – 14.94	3.72±0.84	0.24
Bb-Ea18	9.46	8.34 – 11.93	3.88±0.8	0.59	11.75	9.82 – 18.13	3.80 ±0.9	0.45
Bb-Am4	6.63	6.05 – 7.30	4.40±0.6	4.42	10.01	8.75 – 13.02	3.98±0.83	0.61
Bb-Am5	7.43	6.86 – 8.13	5.38±0.81	0.18	8.20	7.52 – 9.21	5.05±0.83	0.14
Bb-Am8	8.31	7.49 – 9.64	4.09±0.64	0.14	12.13	9.87 – 21.1	3.41±0.86	0.86
Bb-Am20	8.32	7.63 – 9.39	5.07±0.83	0.51	11.81	9.78 – 18.85	3.58±0.87	0.54
Ma-Am1	7.05	6.44 – 7.76	4.75±0.76	1.82	10.58	8.97 – 15.43	3.38±0.8	0.24
Bb-Bo3	8.53	7.69 – 9.98	4.27±0.79	0.17	11.22	9.45 – 16.77	3.62±0.85	0.63
Bb-Bo9	6.79	6.22 – 7.41	5.01±0.76	0.1	13.28	10.31 –29.88	2.92±0.84	0.09
Bb-Bo10	6.66	6.08 – 7.92	4.97±0.77	1.0	9.61	8.33 – 11.98	4.62±0.89	0.15
Bb-Bo16	8.05	7.36 – 9.08	4.78±0.8	1.17	11.31	9.29 – 19.01	3.03±0.79	0.34
Ma-Bo2	9.16	7.9 – 12.46	3.02±0.73	0.37	11.51	9.59 – 18.02	3.51±0.85	0.71
Ma-Bo3	9.67	8.42 – 12.72	3.60±0.78	1.43	11.65	9.99 - 16.55	4.8±1.08	0.93

Bo9, Bb-Bo10 and Bb-Bo16. This isolates were isolated from different district and location which mean that there are a variation between the isolates from the specific location in their virulence against tested larvae. The most virulent isolates toward *G. mellonella* larvae was Bb-Ea7 with LT_{50} value 6.28 days while the most virulent isolate against S. littoralis larvae was Bb-Mo12 with LT_{50} value 7.81 days. The LT_{50} range between 6.28 – 11.21 days for tested isolates against G. mellonella while the range of LT_{50} for the native isolates against S. littoralis were 7.81 – 13.28 days. We also can observe that there is no any isolate have a higher virulence toward *S. littoralis* in compare with *G. mellonella* from the tested isolates.

Data in Table (2) show the cumulative mortality percentage of S. littoralis and G. mellonella larvae after treatment with different concentration of selected native and exotic EPF isolates. The data show that mortality percentage was in a linear relationship with conidial concentration, mortality increased as the applied conidia suspension increase in all tested EPF isolates. The higher mortality rate 96.5% obtained with Bb-Mo12 isolate against G. mellonella larvae at 109 conidia ml-1. In

Table (2): LC_{50} and their 95% confidence limit , slope± SE and X^2 for native and exotic EPF isolates against two lepidopteron larvae *S. littoralis* and *G. mellonella*

Fungi isolate	G. mellonella				S. littoralis			
	LC_{50} spore ml^{-1}	Confidence limit (95%)	Slope ± SE	X^2	LC_{50} spore ml^{-1}	Confidence limit (95%)	Slope ± SE	X^2
Bb-Am5	$1.6x10^7$	$6.6x10^6$ – $3.7x10^7$	0.76± 0.14	1.25	3.9 x 10^7	$1.5x10^7$ – $1.1x10^8$	0.60± 0.12	1.75
Bb-Bo10	$5.0x10^6$	$1.8x10^6$ – $1.1x10^7$	0.79± 0.14	0.32	1.1 $x10^8$	$4.3x10^7$ – $3.8x10^8$	0.61± 0.12	0.67
Bb-Mo12	$1.3x10^6$	$1.5x10^5$– $3.3x10^6$	0.74± 0.2	0.15	1.1 $x10^7$	$2.3x10^6$ – $3.4x\ 10^7$	0.48± 0.11	0.77
Bio-power	$1.1x10^8$	$2.8x10^7$– $1.0x10^9$	0.40± 0.11	0.02	2.6 $x10^8$	$7.1x10^7$ – $3.2x\ 10^9$	0.43± 0.11	0.1
Bio-magic	$1.1x10^7$	$3.1x10^6$ $2.9x10^7$	0.57± 0.12	0.06	2.5 $x10^7$	$6.8x10^6$– $8.5x10^7$	0.49±0. 11	0.32

Table (3): Corrected cumulative mortality percentage (mean±SE) for selected native and exotic EPF isolates toward two lepidopteron larvae S. littoralis and G. mellonella

Fungi isolate	Insect host	% corrected cumulative mortality (mean ±SE)*			
		$1x10^6$	$1x10^7$	$1x10^8$	$1x10^9$
Bb-Am5	*G. mellonella*	19.88±3.33[a]	39.88±3.33[a]	73.22±6.66[a]	89.88±3.33[a]
	S. littoralis	16.55±5.77[a]	29.88±8.82[a]	66.55±5.77[a]	73.22±6.67[a]
Bb-Bo10	*G. mellonella*	29.88±3.33[a]	53.22±3.33[a]	83.22±3.33[a]	93.22±3.33[a]
	S. littoralis	9.88±3.33[b]	23.22±6.67[b]	53.22±6.67[b]	66.55±5.77[b]
Bb-Mo12	*G. mellonella*	46.55±10.0[a]	69.88±3.33[a]	89.88±3.33[a]	96.55±0.0[a]
	S. littoralis	26.55±5.77[a]	49.88±3.33[b]	69.88±3.33[b]	76.55±5.77[b]
Bio-power	*G. mellonella*	19.88±3.33[a]	33.22±3.33[a]	46.55±5.77[a]	63.22±3.33[a]
	S. littoralis	13.21±3.33[a]	26.55±5.77[a]	43.22±3.33[a]	56.55±5.77[a]
Bio-magic	*G. mellonella*	29.88±6.67[a]	46.55±5.77[a]	69.88±3.33[a]	83.22±3.33[a]
	S. littoralis	23.22±3.33[a]	39.88±3.33[a]	63.22±6.67[a]	73.22±3.33[a]

*** For each EPF isolates Means within column followed with the same letter are not significance difference at P ≤ 0.05**

Data in Table (2) show the cumulative mortality percentage of *S. littoralis* and *G. mellonella* larvae after treatment with different concentration of selected native and exotic EPF isolates. The data show that mortality percentage was in a linear relationship with conidial concentration, mortality increased as the applied conidia suspension increase in all tested EPF isolates. The higher mortality rate 96.5% obtained with Bb-Mo12 isolate

against *G. mellonella* larvae at 10^9 conidia ml^{-1}. In addition to, we can observe that there are a significance difference in the mortality percentage between *S. littoralis* and *G. mellonella* larvae (at $p < 0.05$) within isolate Bb-Bo10 at all tested concentration while, in Bb-Mo12 isolate the significance difference in mortality percentage in both tested larvae presence in higher three concentration 10^7, 10^8 and 10^9 conidia m^{-1}, no significance in mortality between both larvae in Bb-Am5, Bio-power and Bio-magic isolate.

Table (4): Analysis of variance of the effect of host species and entomopathogenic fungi isolates and EPF concentration on the corrected cumulative mortality percentage of the host larvae.

Source of variation	df	SS	MS	F	P-value
Host species (A)	1	2773.89	2773.89	84.30	<0.0001
EPF isolates (B)	4	4039.21	1009.80	30.69	<0.0001
EPFconcentration (C)	3	21535.01	7178.34	218.15	<0.0001
(A) X (B)	4	986.14	246.53	7.49	<0.0001
(A) X (C)	3	88.55	29.52	.90	0.4465
(B) X (C)	12	712	59.41	1.81	0.0611
(A) X (B) X (C)	12	77.14	6.44	0.20	0.998
Error	80	2632	32.90		

[a] **df, degree of freedom; SS, sum of squares; MS, mean square; *highly significant (*P* < 0.05).**

Data in Table (3) show the LC_{50} value of selected native and exotic EPF isolates and revealed that G. mellonella was more susceptible that S. littoralis for all tested isolates. In addition, the native isolate Bb-Mo12 and Bb-Bo10 were higher virulence in compare with exotic isolates against *G. mellonella* larvae with LC_{50} $1.3x10^6$ and $5.0x10^6$ conidia ml^{-1} respectively, while the lower virulent isolates was Bio-power with $1.1x10^8$ and $2.6\ x10^8$ conidia ml^{-1} for G. mellonella and S. littoralis larvae respectively.

The analysis of variance (ANOVA) results summarized in Table (4) show highly significant effect of host larvae species ($P < 0.0001$) and EPF isolates ($P < 0.0001$) in addition to their interaction which mean that the effect of host larvae species depend on the EPF isolate and vice versa. Also data show highly significant effect of EPF concentration ($P < 0.0001$) while the interaction of EPF concentration with Host species or EPF isolate or both were not significant.

DISCUSSION

The occurrence of *B. bassiana* and *M. anisopliae* in Egypt, confirms their reported widespread distribution in soils worldwide (Vänninen 1996; Bidochka et al. 1998) in addition to, they were previously isolated from Egypt by El-Husseini et al., 2003 who isolate three species of EPF *B. bassiana*, *M. anisopliae* and *Pacilomyces lilaceous*. From three governorate Giza, Dakahlya and Kafr El-shikh using the insect bait technique they found the recovery rate of EPF from collected soil sample was 1.07% with total 16 isolates. In other work from Egypt Sabry et al., 2011 isolate *B. bassiana* and *M. anisopliae* from soil sample collected from El-Behira Governorate.

The entomopathogenic fungi, used in the present work caused considerable mortality effects against the tested larval of *S. littoralis* and *G mellonella* which agree with Ashraf and El-Katatny (2007) who stated the competency of the entomopathogenic fungi, *B. bassiana*, *A. flavus* Link and *T. harzianum* to be used in the biocontrol regime against the Egyptian cotton leaf worm, *S. littoralis.* In addition several workers report a result agree which our finding such Zayed, 2003, who test the pathogenicity of two native isolate of B. bassiana and found virulence variation in the LT_{50} and LC_{50} toward *G. mellonella* larvae

The present study show variability in the pathogenicity of tested fungal isolates towards *G. mellonella* and *S. littoralis* larvae. This variation among isolates could be related to, the attachment way of spore into insect cuticle, the speed of germination of the conidia of each isolate, the variation between isolate in the activities of extracellular hydrolytic enzymes or variation in the amount produce of all or some of the infection catalyzing enzymes (Momein, 2010). Perkul and Grula, 1979 on a study of comparing between the development of high and low virulent isolate of *B. bassiana* recognize the difference in the growing manner of the fungal isolates over the cuticle surface of the host which indicate the effect of growing manner in the isolate virulence toward a host insect in addition

The variation in pathogenicity of an isolate toward different insect host may be due to the variation in host immune system and the recognition ability (Chandler et. al., 1993), extracellular subtilisin-like chymoelastase designated protease and other components are considered one of the main pathogenicity determinants for entomopathogenic fungi (St. Ledger et al. 1987; Castillo et al. 2000). The potential activity of entomopathogenic fungal isolate may probably be due to the combination of enzymatic, volatile and non-volatile antibiotic activities, which are thought to be closely related in virulence factors for some entomopathogenic fungi and play a role in their mycoinsectisides against the target insects (Fan et al. 2007).

From the obtaining results, it could be concluded that the soil sample contain a variety of entomopathogenic fungi which differ in their virulence toward different insect host and concentration dependence. A promising native isolates in the control of insect pest could be compete the commercial product of entomopathogenic fungi in their virulence and pathogenicity.

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الكفاءة الأبادية لعزلا محلية من الفطريات الممرضة للحشرات على يرقات ديدان الشمع ودودة ورق القطن

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تعتبر الفطريات الممرضة للحشرات من عوامل المكافحة البيولوجية للأفات الحشرية والتي زاد الاهتمام بها في الفترة الأخيرة بهدف البحث عن بدائل للمبيدات الكيماوية لتقليل الاضرار الناتجة عن استخدامها بكثافة في البيئة. في هذه الدراسة تم تقييم كفائة ٢٤ عزلة فطرية ممرضة للحشرات تم عزلها من البيئة المحلية بمحافظة الإسكندرية وتم التعرف عليه ميكروسكوبيا ووجد ان ٢١ عزلة هي فطر *Beauveria bassiana* في حين ان ٣ عزلات هي فطر *Metarhizium anisopliae*. تم عمل تقييم أولى لهذه العزلات الفطرية باستخدام تركيز 10^8 جرثومة مل$^{-1}$ على يرقات العمر الثالث لدودة ورق القطن ويرقات العمر الرابع من ديدان الشمع بالإضافة الا اجراء تجربة مقارنة لدرسة علاقة تركيز الجراثيم بنسبة الموت وذلك على ثلاث عزلات محلية وعزلتين من الخارج هم فطر *B. bassiana* والموجود تحت اسم تجارى Bio-power وفطر *M. anisopliae* والموجود تحت الاسم التجارى Bio-magic وقد أوضحت النتائج وجود اختلافات في القدرة الامراضية بين العزلات المحلية وان يرقات ديدان الشمع اكثر حساسة من ديدان ورق القطن لكل العزلات المختبره. وكانت قيمة الوقت اللازم لقتل ٥٠ % من العشيرة المختبرة (LT_{50}) للعزلات على يرقات ديدان الشمع ٦.٢٨ – ١١.٢١ يوم في حين كانت تتراوح بين ٧.٨١ – ١٣.٢٨ يوم على ديدان ورق القطن. أظهرت النتائج أيضا معنويه لـ ١٣ عزله في قيمة الـ LT_{50} على يرقات ديدان الشمع بالمقارنة مع ديدان ورق القطن. كما أظهرت النتائج المتحصل عليها في تجارب علاقة التركيز بنسبة الموت أن ارتفاع نسبة الموت في اليرقات المعاملة يزداد بشكل خطى مع زيادة تركيز الجراثيم في جميع العزلات المحلية والغريبة وكانت العزلة المحلية Bb-Mo12 أعلى العزلات كفائه بقيمة LC_{50} على ديدان الشمع $1.3x10^6$ جرشومة مل$^{-1}$ وعلى ديدان ورق القطن $1.1x10^7$ جرثومة مل$^{-1}$ في حين كانت عزلة Bio-powerالأقل في الكفائة حيث أعطت اكبر قيمه LC_{50}. من ذلك يمكن ان نقول ان البيئة المحلية تحتوى على عزلات تتنوع فيما بينا من حيث الكفاءة الأبادية التي تختلف أيضا باختلاف العائل الحشري الامر الذى يدعونا الى إمكانية زيادة الاعتماد على مثل هذه العزلات الفطرية واستخدامها كعوامل مكافحة بيولوجية بهدف تقليل الاعتماد على المبيدات الكيماوية في عملية مكافحة الافات الحشرية.