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Evaluation of some isolates of Entomopathogenic fungi on some insect pests infesting potato Crop in Egypt

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Abstract : The present studies were evaluation of some entomopathogenic fungi, *Beauveria bassiana Metarhizium anisopliae* and *Verticilliun lecanii* on *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae*. Each insect species was treated with entomopathogenic fungi, *Beauveria bassiana Metarhizium anisopliae* and *Verticilliun lecanii* at the concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml. *Beauveria bassiana* was more effective against *Agrotis ipsilon* and *Spodoptera littoralis* and less effective against *Myzus Persicae*. *Verticilliun lecanii* was more effective against *Myzus Persicae* and less effective against *Agrotis ipsilon* and *Spodoptera littoralis. Metarhizium anisopliae was less* effective against *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae*. Three concentrations of *B. bassiana* were tested against *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae*. LC_{50} were 2.3 x 10^4 spores / ml. 2.4 x 10^4spores / ml. and 2.7 x 10^4spores / ml. respectively. The same concentrations of *M. anisopliae* were tested against *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae*. LC50 were 2.5 x 10^4spores / ml. 1.5 x 10^4spores / ml. and 2.1 x 10^4spores / ml. respectively. The same concentrations of *Verticilliun lecanii* were tested against *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae*. LC50 were 3.4 x 10^4spores / ml. 2.7 x 10^4spores / ml. and 1.5 x 10^4spores / ml. respectively.

Keywords: Evaluation, entomopathogenic fungi, *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae* , potato Crop.

1. Introduction

The Potatoes crop in Egypt grown through the winter months and harvested in spring. In Egypt cultivated 200 thousands feddans* it produced Two millions Tons.

It cultivated from mid August until mid February. Potatoes are susceptible to several insects such as cut-worms, leaf hoppers, flea beetles, Aphids and white fly. Entomopathogenic fungi are considered by some entomologists to be the best candidates for the control of aphids (Latge and Papierok, 1988, [1, 2,] and numerous accounts of cereal aphids killed by entomopathogenic fungi have been documented in Europe[4, 5, 6, 7] and south America [8]. Also, many species of entomopathogenic fungi can kill aphids, including *Conidiobolus obscures*

(Hall& Dunn) Remaudiere & Keller, *Erynia neoaphidis* Remaudiere& Hennebert, *Verticilium lecanii* (Zimmerman) Viegas, various species of *Beauveria*, and *Paecilomyces farinosus* [9, 10, 11, 12, 13,14, 15, 16, 17, 18].

In Egypt, some studies revealed the effect of entomopathogenic fungi on the population dynamics of some pests such as: *Aphis craccivora* [19, 20] cereal aphids 21, [22, 23, 24] *Bemisia tabaci* (Abdel-Raheem, *et al* 2009) and *Spodoptera littoralis*, *S. exigua* and nymphs of *Aphis craccivora*[25].

***feddan = 4200 m^2.**

The present studies were aimed to evaluation of some entomopathogenic fungi, *Beauveria bassiana Metarhizium anisopliae* and *Verticilliun lecanii* on *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae* on potato Crop in Egypt

Materials and Methods

Rearing technique

The *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae* Strains used in the present studies were taken from laboratory pests and plant protection at the National Research Centre Giza, Egypt.

Entomopathogenic fungi used

Entomopathogenic fungi used in the present studies were *Beauveria bassiana* and *Metarhizium anisopliae.* The fungi were isolated from the *Scrobipalpa ocellatella* and *Cassida vittata* Kafr El-Shikh governorate and *Verticilliun lecanii* isolated from soil, [26, 27.]

Fungi Cultures

Three concentrations of *B. bassiana, M. anisopliae* and *V. lecanii* were (2×10^3, 2×10^4 and 2×10^5 spores / ml.). The Entomopathogenic fungi were grown on peptone media (10g Peptone, 40g Dextrose, 2g Yeast extract 15g Agar and 500 ml. Chloramphenicol and completed to one liter with 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 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 number of conidia, *B. bassiana, M. anisopliae* and *V. lecanii* isolates were propagated on wetted rice. Two Kilogram 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 isolates and incubated at 25 ±2ºC for 15 days. The Conidia were harvested by distilled water and filtered through cheese cloth to reduce mycelium clumps and Tween 80% was added [29].

Preparation of the Concentrations

Conidia of fungal isolates were harvested by rinsing with sterilized 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 mm2). 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, 2×10^3, 2×10^4 and 2×10^5 spores / ml in all isolates.

Treatment Procedures

Three concentrations of each agent and three replicates for each were tested on *Agrotis ipsilon* and *Spodoptera littoralis* larvae and *Myzus Persicae* nymphs to study the effect of these materials on larval and nymphs mortality. B. *bassiana, M. anisopliae* and *V. lecanii* were prepared with concentrations of (2×10^3, 2×10^4 and 2×10^5 spores / ml). The larvae were fed with untreated leaves when needed. A similar method of experiment was performed to estimate the effect of the three entomopathogenic materials on larvae and nymphs.

The leaves were collected from the potato plants, arranged in Petri dishes and infested with larvae obtained from the laboratory colony. Discs were transferred to Petri dishes and larvae in the appropriate instar were placed in the dishes. The bioassay lasted for 10 days and the median lethal concentration ($LC_{50,\ LC\ 90\ \&\ LT50}$) values were obtained by the software computer propane. The larval and nymphs mortality were evaluated daily for 2, 4, 6, 8 an 10 days. The mortality was corrected using Abbott's formula (Abbott, 1925)

$$\text{Corrected Mortality \%} = 100 \times 1 - \frac{\text{Insect population in treated after treatment}}{\text{Insect population in control after treatment.}}$$

Results

Agrotis ipsilon LC_{50} and LC_{90} values was more susceptible to *Beauveria bassiana* than *Metarhizium anisopliae* and *V. lecanii*, where LC_{50} and LC_{90} were 2.3 x 10 4, 0. 1 x 10^5 spores / ml. , 2.5 x 10^4, 1.3 x 10^5 spores / ml. and3.4 x 10^4, 2.5 x 10^5 spores / ml., respectively. The LT_{50}s were calculated as 5.0, 5.5 and 6.5days for the three respective fungi, indicating the superiority of *Beauveria bassiana* over *Metarhizium anisopliae* and *V. lecanii*(table 1).

Spodoptera littoralis LC_{50} and LC_{90} values was more susceptible to *Metarhizium anisopliae* than *Beauveria bassiana* and *V. lecanii*, where LC_{50} and LC_{90} were 1.5 x 10^4, 0. 0.5 x 10^5 spores / ml. , 2.4 x 10 4, 0. 2 x 10^5spores / ml. and 2.7 x 10^4, 2.4 x 10^5spores / ml., respectively. The LT_{50}s were calculated as 4.5, 5.6 and 6.4days for the three respective fungi, indicating the superiority of *Metarhizium anisopliae* over *Beauveria bassiana* and *V. lecanii*(table 1).

Myzus Persicae LC_{50} and LC_{90} values confirmed that *M. Persicae* was more susceptible to *V. lecanii* than *Metarhizium anisopliae* and *Beauveria bassiana*, where LC_{50} and LC_{90} were 1.5 x 10 4, 0.0 1 x 10^5 spores / ml. , 2.1 x 10^4, 1.01 x 10^5spores / ml. and 2.7 x 10^4, 0. 3 x 10^5 spores / ml., respectively. The LT_{50}s were calculated as 4.5, 5.5 and 6.4days for the three respective fungi, indicating the superiority of *V. lecanii* over *Metarhizium anisopliae* and *Beauveria bassiana* (table 1).

Table 1: LC_{50}, LC_{90} and LT_{50} (days) values of *Agrotis ipsilon*, *Spodoptera littoralis* and *Myzus Persicae* treated with *Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii*

Species	Beauveria bassiana			Metarhizium anisopliae			Verticillium lecanii		
	LC_{50}	LC_{90}	LT_{50}	LC_{50}	LC_{90}	LT_{50}	LC_{50}	LC_{90}	LT_{50}
Agrotis ipsilon	2.3 x 10^4	0. 1 x 10^5	5.0	2.5 x 10^4	1.3 x 10^5	5.5	3.4 x 10^4	2.5 x 10^5	6.5
Spodoptera littoralis	2.4 x 10^4	0. 2 x 10^5	5.6	1.5 x 10^4	0.5 x 10^5	4.5	2.7 x 10^4	2.4 x 10^5	6.4
Myzus Persicae	2.7 x 10^4	0. 3 x 10^5	6.4	2.1 x 10^4	1.01 x 10^5	5.5	1.5 x 10^4	0.0 1 x 10^5	4.5

Table 2 show cumulative mortality percentage of *A. ipsilon* maximum mortality percentages larvae treatment with *B. bassiana, M. anisopliae* and *V. lecanii* were recorded 10 days after where the mortality were 35.2, 38.1and 40.2% for *B. bassiana* at the tested concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml., respectively, 33, 35 and 37 % for *M. anisopliae* at the tested concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml., respectively and25, 27 and 35 % for *V. lecanii* at the tested concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml., respectively.

Table 2: The Cumulative mortality percentages of *A. ipsilon* treated with *B. bassiana, M. anisopliae* and *V. lecanii*.

Fungi	Conc. spores/ml.	Mortality percentages of *A. ipsilon* indicated days				
		2	4	6	8	10
B. bassiana	2 x 10^3	2±1	20±2.2	25±1.5	31±1.9	35.2±2.1b
	2 x 10^4	4±1.5	23±2.2	27±1.5	32±1.1	38.1±2.3b
	2 x 10^5	5±2.5	28±1.5	2±1.23	39±1.9	40.2±2.7a
M. anisopliae	2 x 10^3	1.7±1	18 ± 2.1	3±12	25±4	33±2a
	2 x 10^4	2 ±1.3	20 ± 2	4±22	30±1	35±3 b
	2 x 10^5	2.2±1.5	25±2.2	27±4	35±2	37 ±2b
V. lecanii	2 x 10^3	1±1	17±1	20±1.5	23±2	25±2 c
	2 x 10^4	2±1.2	18±2	22±2	25±3	27±2.3c
	2 x 10^5	2±1.5	22±3	25±3	33±2	35±2.1b

Spodoptera littoralis

The result in table 3 show the cumulative mortality percentages started low on the 2nd day. The Percent mortality of *Spodoptera littoralis* was increased, till the maximum was recorded on 10th days of observation, where the mortality percentages were recorded 35.2, 38 and 40.2% for *B. bassiana* respectively 38, 40 and 45 % for *M. anisopliae* and 23, 29 and 38 % for *V. lecanii* at the tested concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml., respectively.

The *S. littoralis* was more susceptible to infection with *M. anisopliae* than *B. bassiana and V. lecanii*.

Table 3: The mortality percentages of *S. littoralis* treated with *B. bassiana, M. anisopliae* and *V. lecanii*.

Fungi	Conc. spores/ml.	Mortality percentages of *A. ipsilon* indicated days				
		2	4	6	8	10
B. bassiana	2 x 10^3	1±1	19 ± 2	25±1	28±1.9	35.2±2 c
	2 x 10^4	2±2	20± 2.3	27±2	32±1.1	38.1±2c
	2 x 10^5	3±1	25 ±1	1±23	39±1.9	40.2±1b
M. anisopliae	2 x 10^3	2.1±1	22 ± 2	6±12	35±4	38±1a
	2 x 10^4	4 ±2	24 ± 2	8±22	38±1	40±3 b
	2 x 10^5	5±1	26 ± 2	35±4	40±2	45 ±2a
V. lecanii	2 x 10^3	0.0	17±2	19±3	22±2	23±2 d
	2 x 10^4	1±1	18±2.5	23±2	26±3	29±2 d
	2 x 10^5	2±1	22±3.3	27±1	35±2	38±2c

Myzus Persicae

The results in table 3 showed that the cumulative mortality percentages began with low on the 2nd day after treatment. Then the Percent mortality of *Myzus Persicae* was increased, till the maximum was recorded on the 10th days of observation, where the mortality percentages were recorded 25.2, 31 and 33% for *B. bassiana* respectively 28, 32 and 38 % for *M. anisopliae* at the tested concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml., respectively and 37, 41 and 47 % for *V. lecanii* at the tested concentrations of 2 x 10^3, 2 x 10^4 and 2 x 10^5 spores / ml., respectively.

The *Myzus Persicae* was more susceptible to infection with *V. lecanii* than *M. anisopliae and B. bassiana* (Table 4).

Table 4: The Cumulative mortality percentages of *Myzus Persicae* treated with *B. bassiana, M. anisopliae* and *V. lecanii* .

Fungi	Conc. spores/ml.	Mortality percentages of *A. ipsilon* indicated days				
		2	4	6	8	10
B. bassiana	2 x 10^3	0.0	17 ± 1	19±2	20±1	25±2 d
	2 x 10^4	2±1	19± 1	21±1	22±1	31±1c
	2 x 10^5	2 ±1	20 ±1	3±22	25±2	33±1c
M. anisopliae	2 x 10^3	2 ±1	18 ± 1	21±1	23±1	28±2d
	2 x 10^4	2 ±2	19± 2	22±2	25±2	32±1 b
	2 x 10^5	3 ±1	21 ±1	4±22	30±1	38 ±1b
V. lecanii	2 x 10^3	2 ±1.1	20±1	23±1	25±2.1	37±1 b
	2 x 10^4	3±2	22±2	25±1	28±2	41±1 a
	2 x 10^5	4±1	27±2	30±1	35±1	47±1a

Discussion

Beauveria bassiana, Metarhizium anisopliae and *Verticilliun lecanii* are naturally fungi that are found in the soil of most fields [29, 30, 31, 32, 34, & 35]. These fungi are entomopathogenic which causing disease to insects. Fungal infection begins when conidia (asexual spores, the seeds of a fungus) attach to insect's cuticle, the spores germinate and penetrate the insect's skin and enter the host.

B. bassiana was the most effects on the larvae of *A. ipsilon* than *M. anisopliae* and *V. lecanii*, but *M. anisopliae* was the most effects on the larvae of S. *littoralis* than *B. bassiana* and *V. lecanii*.

V. lecanii was the most effects on the nymphs of *M. Persicae* these results according with.[36, 37, 38, 39.]

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