Impact of Entomopathogenic Fungi *Beauveria Bassiana* and Isaria *fumosorosea* on Cruciferous Aphid *Brevicoryne Brassicae* L.

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ABSTRACT: An experiment was conducted to evaluate the role of two entomopathogenic fungi: *Beauveria bassiana* (Bals.) and *Isaria fumosorosea* (Wize) against the adults of cruciferous aphid (cabbage aphid), *Brevicoryne brassicae* L. under laboratory conditions at plant protection institute, Sharkia Branch. Three tested concentrations (10^5, 10^6 and 10^7 spores/ml) of *B. bassiana* formulations were used against aphid *B. brassicae*. Mortality percentage after 7 days of application showed 35.55, 46.66 and 64.44%, respectively. The obtained results revealed that LC_{50} was 1.1×10^6 spores/ml and LC_{90} was 3.4×10^9 spores/ml. While, Three tested concentrations (10^6, 10^7 and 10^8 spores/ml) of *I. fumosorosea* formulation were used against the same aphid. Mortality percentage after 7 days of application showed 37.77, 60.0 and 73.33%, respectively. The obtained results revealed that LC_{50} was 3.9×10^6 spores/ml and LC_{90} was 2.1×10^9 spores/ml.

Keywords: Biological control, Entomopathogenic fungi, *Beauveria bassiana, Isaria fumosorosea*, *Brevicoryne brassicae*

INTRODUCTION

The aphid *Brevicoryne brassicae* L. is a major pest on cruciferous plants, in several parts of the world and also in Egypt especially on cabbage cauliflower (Horn 1989 and Saleh 2004 and 2014). Entomopathogenic fungi are natural enemies of insects and arachnids and they contribute in the regulation of their host population. In agriculture, they have been observed to cause mortality in pest's population (El-husseini *et al*., 2010). Several species of entomopathogenic fungi can be used as natural enemies against many pests, including *Onidiobolus obscures*, *Erynid neophidis*, *Verticillium lecanii*, various species of *Beauveria*, and *Isaria fumosorose*a (Wize) (Roberts and Yendol 1971, Samson *et al.* 1988 and Hayden *et al.* 1992).

MATERIALS AND METHODS

1. Culture of *B. brassicae*

The cabbage's aphid *B. brassicae*, was reared on cabbage leaves under laboratory conditions 25±1°C, 65±5 RH% and 12 hr photoperiod. The cabbage leaves that were used for laboratory evaluation contained a midrib in order to survive for a long period of time. Aphids were taken by a fine brush and located on cabbage leaves. The leaves were incubated under the same laboratory conditions 25±1°C, 65±5 RH% and 12 hr, photoperiod. After 48 hr, three leaves were picked up and put in a plastic Petri plates on filter papers that saturated daily with water.

2. Fungal inocula

Spores of fungal isolate were harvested by rinsing with sterilized water containing 0.005% Tween80 from 7days old culture (Dox medium grown at 25±1°C for *B. bassiana* and *I. fumosorose*a isolates). The suspensions were filtered through cheesecloth to reduce mycelium clumping. The spores were counted in the suspensions using a haemocytometer. The concentrations were adjusted to 10^5, 10^6 and 10^7 for *B. bassiana* and 10^6, 10^7 and 10^8 for *I. fumosorose*a.

3. Experimental work

Studies regarding the effect of the fungus on the infected leaves of cabbage were applied on three replicates each consists of fifteen individuals of *B. brassicae* on cabbage leaves. Leaves were sprayed with two ml of spore suspension and the control was treated with two ml of sterilized water containing 0.005 % tween80 only. The treatments and control were incubated for 7 days under laboratory conditions (25±1°C, 65±5 RH% and 12 hr photoperiod). Larval mortality was observed after 1, 3, 5and 7 days. LC_{50} and LC_{90} and slop values were calculated after 5 and 7 days according to Finny (1971). The presented results are means of each treatment.

The obtained results were statistically analyzed by using Costat (2005) computer program.

RESULTS AND DISSCUTION

1. Laboratory evaluation of the efficiency of entomopathogenic fungi on *B. brassicae*

1.1. The entomopathogenic fungi *B. bassiana*:

Laboratory evaluation: Data given in Table (1) shows the efficacy of *B. bassiana* spores suspension on nymph instars of cabbage aphid *B. brassicae* after application with different concentrations of *B. bassiana* spores under laboratory conditions of (25±1°C, R.H. 65±5%). The concentrations were adjusted to 10^5,10^6 and 10^7 spores/ml. Mortality percentages after 5 days of application showed 22.22%, 28.88% and 42.22% and after 7 days showed 35.55%, 46.66% and 64.44%, respectively.

Data given in Table (3) clarified the LC_{50} and LC_{90} values of *B. bassiana* spores/ml after 5 and 7 days of application on nymph instars of cabbage aphid, *B. brassicae*. The obtained results revealed that after 5 days LC_{50}: 5.8×10^7 spores/ml and LC_{90}: 1.7×10^{12} spores/ml (Fig. 1) and after 7 days LC_{50}: 1.1×10^6 spores/ml and LC_{90}: 3.4×10^9 spores/ml (Fig. 2).

Entomopathogenic fungi have been observed to cause mortality in pest population and thus, investigated for their potential as biological control agents (Hesketh *et al*., 2008) or successfully developed as biocontrol agent for a number of different pests, including aphids (Shah and Pell, 2003; De Faria and Wraight, 2007).

Time-dose dependent mortality response experiments were designed as a measure of mortality of different fungal isolates against aphids. The mortality observed was low on day 1 and 2, after the treatment, the mortality then dramatically increased from day 7 to 9. The mortality in infected aphids with fungal isolates increased with increase in spore concentration of conidial suspensions and exposure time. The susceptibility of target insect to fungal infection is dose dependant (Liu and Chen, 2002; Wright *et al*., 2005).

Meanwhile, Akmal *et al*. (2013) showed that the maximum mortality 100% of *B. bassiana* on *B. brassicae* was obtained at 7th day post treatment at a concentration of 1×10^8, while minimum mortality of 99.2% with treatment of 1×10^6. In contrast to this no mortality was recorded in control. The value of LC_{50} 6.28×10^5 showed that the 50% mortality was obtained at 3rd day of treatment.

On the other hand, Akbari *et al*. (2014) in Iran showed that the adult aphids *B. brassicae* were treated with fungal concentrations of 1×10^3 to 1×10^7 spores/ml. The lowest LT_{50} was obtained at 7.67 days for Iran 429C (*B. bassiana*) isolate at concentration 1×10^7 spores/ml.

1.2. The entomopathogenic fungus *I. fumosorosea*:

Laboratory evaluation: Data given in Table (2) shows the efficacy of *I. fumosorose*a spores suspension on nymph instars of cabbage aphid *B. brassicae* after application with different concentrations of *I. fumosorose*a spores under laboratory conditions of (25±1°C,R.H. 65±5%). The concentrations were adjusted to 10^6,10^7 and 10^8 spores/ml. Mortality percentage values after 5 days of application showed 24.44, 40 and 51.11%, and after 7 days showed 37.77,60 and 73.33%, respectively.

Data given in Table (4) clarified the LC_{50} and LC_{90} values of *I. fumosorose*a spores/ml after 5 and 7 days of application on nymph instars of cabbage aphid, *B. brassicae*. The obtained results revealed that after 5 days LC_{50}: 7.1×10^7 spores/ml and LC_{90}: 2.7×10^{11} spores/ml (Fig. 3) and after 7 days LC_{50}: 3.9×10^6 spores/ml and LC_{90}: 2.1×10^9 spores/ml (Fig. 4).

Asi *et al.* (2009b) showed that the concentration of the entomopathogenic fungus *I. fumosorose*a affected the mortality of cabbage aphids *B. brassicae* differently. The LC_{50} of this fungus against the adults of aphids 7 days after conidial treatment was 2.22×10^6 conidia/ ml. this fungus resulted in mortality of 5.83% on the 1st day of treatment. The mortality then increased on days

5-7. The cumulative mortality caused by the fungus after 7 days of treatment was 18.14-90.82%. The mortality increased with the increase in the spores concentration and exposure time.

Also, Asi *et al.* (2009a) showed that the concentration (1×10^6 conidia/ml) of the entomopathogenic fungus *I. fumosorose*a resulted in 20.17% mortality of adults of cabbage aphids *B. brassicae* three days after conidial treatment. The mortality percentage of *B. brassicae was* 48.40% and 68.57% after 5 and 7 days of conidial treatment. However, virulence potential of entomopathogenic fungi against target aphid varies from isolate to isolate and from strain to strain. The susceptibility of the same aphid species may vary to different fungal strains. Even biotypes or different colons of the same aphid species may have varying susceptibility to fungal infection (Ferrari *et al.*, 2001 and Blanford *et al.*, 2003). *V. lecanii*, *M. anisopliae* and *P. fumosorose*us can effectively control aphids (Asi *et al*., 2009a).

CONCLUSION

The two entomopathogenic fungi: *B. bassiana* and *I. fumosorose*a have efficiency in controlling cabbage aphid *B. brassicae* and can be used as biological control agents against *B. brassicae*.

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Table (1): Mortality percentages of nymph instars of *B. brassicae* after application with different concentrations of *B. bassiana* spores suspension under laboratory conditions (25±1°C, R.H. 65±5%) during winter season of 2012/2013.

Concentration	After 1 day			After 3 days			After 5 days			After 7 days		
	Life	Dead	Mortality %	Life	Dead	Mortality %	Life	Dead	Mortality %	Life	Dead	Mortality %
1×10^5 spores/ml	44	1	2.22	39	6	13.33	35	10	22.22	29	16	35.55
1×10^6 spores/ml	42	3	6.66	36	9	20	32	13	28.88	24	21	46.66
1×10^7 spores/ml	41	4	8.88	34	11	24.44	26	19	42.22	16	29	64.44

Table (2): Mortality percentage values of nymph instars of *B. brassicae* after application with different concentrations of *I. fumosorose*a spores suspension under laboratory conditions (25±1°C, R.H. 65±5%) during winter season of 2012-2013.

Concentration	After 1 day			After 3 days			After 5 days			After 7 days		
	Life	Dead	Mortality %	Life	Dead	Mortality %	Life	Dead	Mortality %	Life	Dead	Mortality %
1×10^6 spores/ml	44	1	2.22	40	5	11.11	34	11	24.44	28	17	37.77
1×10^7 spores/ml	43	2	4.44	37	8	17.77	27	18	40.00	18	27	60.00
1×10^8 spores/ml	41	4	8.88	33	12	26.66	22	23	51.11	12	33	73.33

Table (3): Lethal concentration (LC_{25-99}) of *B. bassiana* spores/ml after 5 and 7 days of application against nymph instar of *B. brassicae* under laboratory conditions (25 ± 1°C, R.H. 65± 5%) during winter season of 2012/2013.

Lethal concentration	Concentration of *B. bassiana* spores/ml						Slope	
	Concentration		Lower limit		Upper limit			
	After 5 days	After 7 days	After 5 days	After 7 days	After 5 days	After 7 days	After 5 days	After 7 days
LC_{25}	258760	18062.3	8837	353.66	874830	81558.6	0.287	0.37
LC_{50}	5.8×10^{7}	1.1×10^{6}	9.5×10^{6}	4.5×10^{5}	9.1×10^{10}	3.5×10^{6}		
LC_{75}	1.3×10^{10}	7.9×10^{7}	2.9×10^{8}	1.5×10^{7}	3.2×10^{17}	5.4×10^{9}		
LC_{90}	1.7×10^{12}	4.3×10^{9}	6×10^{9}	2.2×10^{8}	2.8×10^{23}	7.2×10^{12}		
LC_{95}	3.1×10^{13}	3.3×10^{10}	3.6×10^{10}	1×10^{9}	1×10^{27}	5.5×10^{14}		
LC_{99}	7.4×10^{19}	2.2×10^{12}	1×10^{12}	1.8×10^{10}	5.1×10^{33}	1.9×10^{18}		

Table (4): Lethal concentration (LC_{25-99}) of *I. fumosorose*a spores/ml after 5 and 7 days of application against nymph instar of *B. brassicae* under laboratory conditions (25 ± 1°C, R.H. 65± 5%) during winter season of 2012-2013.

Lethal concentration	Concentration of *I. fumosorose*a spores/ml						Slope	
	Concentration		Lower limit		Upper limit			
	After 5 days	After 7 days	After 5 days	After 7 days	After 5 days	After 7 days	After 5 days	After 7 days
LC_{25}	921610	143230	51058.9	8777.84	2.9×10^{6}	5.3×10^{5}	0.357	0.469
LC_{50}	7.1×10^{7}	3.9×10^{6}	2.5×10^{7}	1.4×10^{6}	7.1×10^{8}	8.1×10^{6}		
LC_{75}	5.4×10^{9}	1×10^{8}	5.9×10^{8}	4.2×10^{7}	3.6×10^{12}	6.6×10^{8}		
LC_{90}	2.7×10^{11}	2.1×10^{9}	8.4×10^{9}	4.1×10^{8}	9.6×10^{15}	7.7×10^{10}		
LC_{95}	2.8×10^{12}	1.2×10^{10}	4×10^{10}	1.5×10^{9}	1×10^{18}	1.4×10^{12}		
LC_{99}	2.3×10^{14}	3.6×10^{11}	7.6×10^{11}	1.7×10^{10}	7.8×10^{21}	3.2×10^{14}		

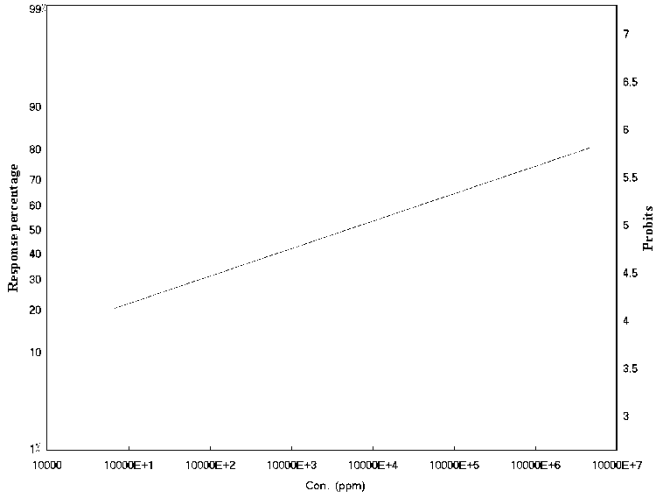

Fig. (1): Concentration mortality probit line of *B. bassiana* spores/ml on nymph instars of *B. brassicae* under laboratory conditions (25 ± 1°C, 65± R.H. 5%) after 5 days.

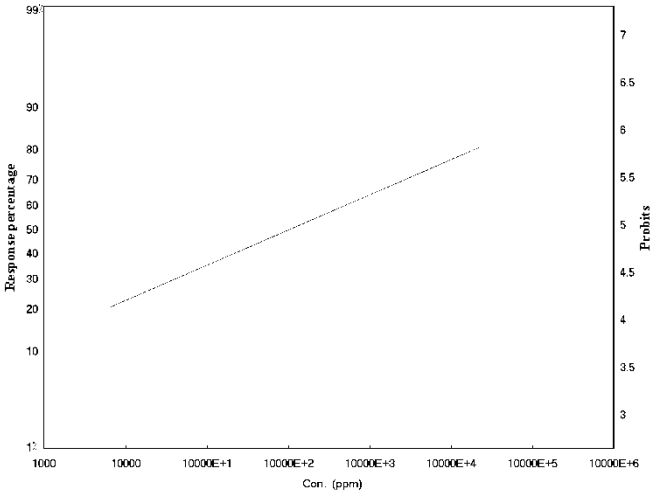

Fig. (2): Concentration mortality probit line of *B. bassiana* spores/ml on nymph instars of *B. brassicae* under laboratory conditions (25 ± 1°C, 65± R.H. 5%) after **7** days.

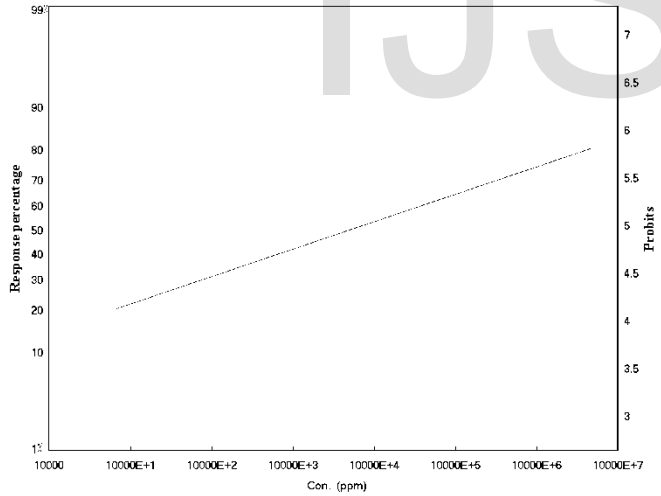

Fig. (3): Concentration mortality probit line of *I. fumosorose*a spores/ml on nymph instars of *B. brassicae* under laboratory conditions (25 ± 1°C, 65± R.H. 5%) after **5** days.

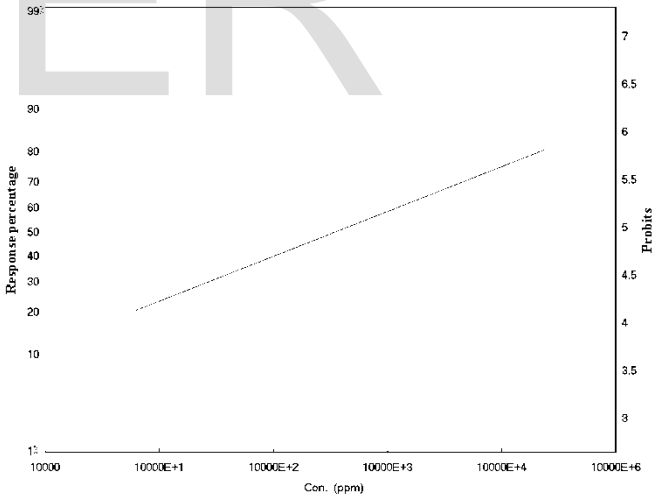

Fig. (4): Concentration mortality probit line of *I. fumosorose*a spores/ml on nymph instars of *B. brassicae* under laboratory conditions (25 ± 1°C, 65± R.H. 5%) after 7 days.