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Impact of Entomopathogenic Fungi on the Aphid, *Brevicoryne brassicae* L. and its Associated Predator, *Coccinella undecimpunctata* L.

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ABSTRACT

Four tested concentrations of Bioranza (*Metarhizium anisopliae*) and Biovar (*Brevicoryne brassicae*) formulations were used against aphid, *Brevicoryne brassicae* L. and its associated the coccinellid predator, *Coccinella undecimpunctata* L. The lowest concentrations of the two formulations (0.5 and 1.0g/L) were relatively safe to the predator's adults, while the highest concentrations (2.0 and 4.0g/L) were injurious. The LC_{50} and the LT_{50} values revealed that the Biovar formulation was more potent and faster in action than Bioranza.

Key Words: *Brevicoryne brassicae*, *Coccinella undecimpunctata*, entomopathogenic fungi, *Metarhizium anisopliae, Beauveria bassiana.*

INTRODUCTION

Brevicoryne brassicae (L.) aphid (Hemiptera: Aphididae) feeds on all cultivated and wild cruciferous plants. The major economic host plants include broccoli, cauliflower, head cabbage and most of the genus *Brassica*. Colonies of this aphid are found on both lower and upper leaf surfaces, leaf stalks and leaf axles as well as in leaf folds of developing heads. Infested seedlings may become stunted and distorted. Continuous feeding on mature plants causes wilting, yellowing and general stunting of the plants (Berry, 1998).

In addition, cabbage aphid is a vector of 23 virus diseases of cruciferae and many diseases of citrus (Toba, 1962). Naturally occurring parasitoides and predators are important factors in regulating population densities. The ladybird beetle, *Coccinella undecimpunctata* L. (Coleoptera : Coccinellidae) is one of the most efficient predators of aphids.

There are many insecticides that are effective against this aphid. The broad-spectrum organophosphate (OP) insecticides have been successfully used for controlling insect pests for many years and many growers used to rely solely on these products one season after another (Berry, 1998). Such repeated usage in many cultivated areas formed an adverse effect on many beneficial insect populations and consequently caused outbreaks of secondary pests such as mites, as well as developing resistance of insecticide. Recently, many entomopathogenic fungi are used as formulations to control many agricultural insect pests. Numerous studies have screened fungi against aphids (Driver *et al.* and 2000; Filotas *et al.*, 2004).

The present study aimed to evaluate the efficacy of two fungal formulations of *Metarhizium anisopliae* (Mechnikov) and *B. bassiana* (Balsamo-Crivelli) against the cabbage aphid, *B. brassicae* and to study their side effect on *C. undecimpunctata* associated with this pest, for nominating the proper efficient concentration against the aphid pest and relatively safe for the predator.

MATERIALS AND METHODES

Entomopathogenic fungi

The commercial formulations of the two entomopathogenic fungi were studied under laboratory conditions (27±2°C and 75±5% RH). Bioronza, the commercial formulation of *M. anisopliae* (usually called the green muscardin) and Biovar, the commercial formulation of *B. bassiana* (called the white muscardin) were manufactured and produced by "The Kingdom of Bahrain, Ministry of Municipalities Affairs and Agriculture Wealth Directorate". The active ingredient of Bioranza is (conidia) 10% formulated as WP. Meanwhile, Biovar active ingredient concentration is $32x10^6$ viable spore/mg as for both formulations. The recommended concentration for both formulations was 200g/100L water. Four concentrations were prepared (4, 2, 1 and 0.5g/L water) for each formulation.

Predator treatments

- Egg

Groups of 25 newly deposited eggs of the predator (collected from the maintained culture in the National Research Centre insect laboratory) were sprayed with a suspension of each fungus formulation at each tested concentration (1ml suspension of each concentration/group); the check was sprayed with water only; confined in glass jars (1 liter volume) on a piece of plant leaf with enough numbers of brassicae aphid. The glass jars were covered with muslin and incubated under laboratory

conditions till hatching. The percentage of hatchability was calculated; the hatched larvae were followed up till the end of the experiment.

- Adult

The same steps mentioned above in case of egg treatment was repeated here with substituting the eggs with 25 newly emerged predator adults. The percentage of mortality as a result of fungi infection were calculated. The cadavers were removed from the jars, then surfacely sterilized in 5% sodium hypochlorite and 75% ethanol solution and rinsed in plenty of sterile distilled water, then left to dry for 48h (Dourou-kpinduo *et al.*, 1995). After drying, they were kept in humid conditions in clean dessicators at room temperature to examine whether they died because of fungus infection or not according to Luz and Fargues (1998).

- Aphid treatment

Groups of 25 individuals of aphid (all instars) (collected from the maintained culture in National Research Centre insect laboratory) were sprayed as previously described the check was sprayed with water only, all were in glass jars (1 liter volume) on a piece of plant leaf. The glass jars were covered with muslin and kept under laboratory conditions. Fresh cabbage leaves were introduced each other day for feeding till the end of the experiment. The percentage of mortality as a result of fungi infection were calculated. Cadavers of the aphids were surface sterilized as mentioned above, then transferred into clean containers till the devolving of mycosis.

Statistical analyses:

Obtained data were subjected to analyses of variance (F-test); Duncan Multiple range was applied to compare differences among means. T-test was applied to compare the differences between the two formulations at each tested concentration. The Abbott's formula (Abbott, 1925) were applied to correct the percentage of mortality to calculate the LC_{50} and LT_{50} values for each fungus using Finney (1952) equation.

RESULTS AND DISCUSSION

Data illustrated in figure (1) show that the corrected percentage of hatchability, as a result of the infection with the two entomopathogenic fungi, was more than 90 and 80% at the lower concentrations (0.5 and 1.0g/L), respectively, that mean that the tested fungi were relatively safe to the coccinelid eggs at such low concentrations. While at the higher concentrations (2 and 4.0g/L), the percentage of hatchability was somewhat affected, it

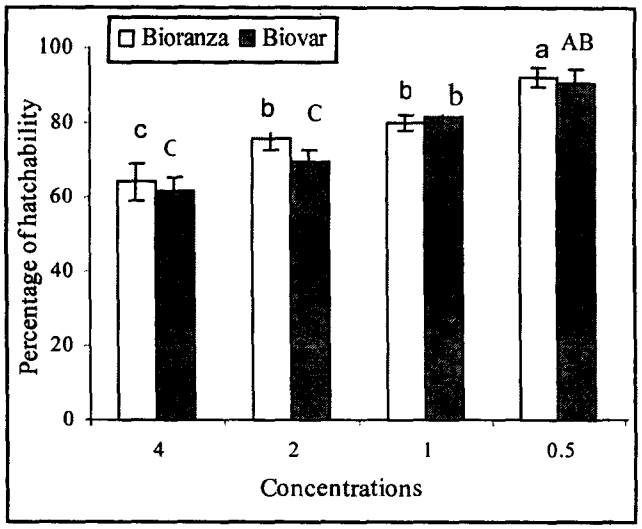

Fig. (1): Corrected percentage of hatchability of *Coccinella* eggs as a result of treatment with the fungi formulations at different concentrations.

was 75.99 and 69.33%, respectively, for Bioranza and 64.00 and 61.33%, for Biovar, respectively.

Adult predator sprayed with the tested concentrations of the two entomopathogenic fungi exhibited a different response, in case of Bioranza treatment; the highest concentration (4.0g/L) gave the highest percent mortality (64.00%) among adults, being significantly different ($P>0.01$) than the lowest concentration (0.5g/L), which induced only 10.00% mortality (Fig. 2). No further mortality was detected after 12 days of application for all tested concentrations.

The corresponding figure of Biovar treatment was nearly the same trend, where the highest concentration (4.0g/L) induced 81.00% mortality, being significantly different of all other tested concentrations ($P>0.01$); while the lowest concentration (0.5g/L) induced only 5.00% mortality. The second (2.0g/L) and the third (1.0g/L) tested concentrations, induced 48.00 and 29.00% mortality, respectively, being significantly different from each other ($P>0.01$). No further mortality was detected after 8 days of application for all tested concentrations. The abovementioned results indicated that, in general, Biovar formulation was more effective than Bioranza against aphids at the first two concentrations (4.0 and 2.0g/L), and vise versa at the lowest concentrations (1.0 and 0.5g/L). The two fungi gave insignificant differences between each other at all tested concentrations ($P>0.05$).

The LC_{50} value of Bioranza and Biovar was 2.699 and 1.906g/L, respectively, showing that the

Table (1): The LC_{50} and slope values for the tested concentrations of the two entomopathogenic fungi on *Beauveria bassiana* and *C. undecimpunctata*.

	Brevicoryne brassicae		*Brevicoryne brassicae*	
	M. anisopliae	*M. anisopliae*	*M. anisopliae*	*B. bassiana*
LC_{50} (g/L)	0.890	0.890	2.699	1.906
Slope	2.814	2.814	1.475	2.603

Table (2): The LT-values for *B. brassicae* and *C. undecimpunctata* treated with fungi products, Bioranza (*M. anisopliae*) and Biovar (*B. bassiana*) at different tested concentrations.

Conc. (g/L)	LT value	*Brevicoryne brassicae*		*Coccinella undecimpunctata*	
		Bioranza	Biovar	Bioranza	Biovar
		Time (in days)			
4.0	LT_{50}	4.140	4.692	6.696	4.802
	Slope	8.964	6.040	1.783	11.221
2.0	LT_{50}	5.857	6.226	13.330	6.656
	Slope	4.822	3.977	1.420	4.528
1.0	LT_{50}	7.535	9.436	28.686	11.598
	Slope	3.951	3.548	0.886	2.400
0.5	LT_{50}	4.758	25.726	123.76	11.846
	Slope	2.991	1.597	0.996	0.998

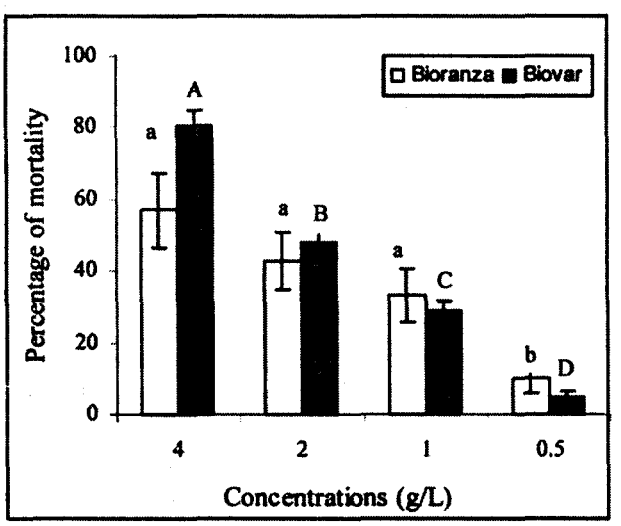

Fig. (2): Corrected mean percentage of mortality of *Coccinella* adults as a result of treatment with the tested entomopathogenic fungi at different concentrations.

Biovar formulation was more effective against aphids than Bioranza, while the LC_{50} of Bioranza was 1.42 times more than Biovar (Table 1).

The least time required for killing 50% (LT_{50}) of predator's adult at the highest concentration (4.0g/L) was 6.696 days for Bioranza, while it was 4.802 days in case of Biovar, that mean that, the latter fungus formulation was faster in inducing the mortality among the predator's adults (Table 2).

The same trend was observed at all tested concentrations (Table 2). It could be concluded that Bioranza formulation was more safe at the lowest concentrations (0.5 and 1.0g/L) towards the predator's adults than Biovar at the same concentrations. While the higher concentrations of the tested formulations were relatively injurious for the important predator, *C. undecimpunctata*.

These results are in accordance with those reported by Haseeb and Murad (1997), who studied the susceptibility of *C. septempunctata* to the entomogenous fungus, *B. bassiana*, they mentioned that the predator was somewhat susceptible the high concentrations of the pathogen. Smith and Krischik (2000) and Ormond *et al.* (2005&2007), reported that *Beauveria* was relatively injurious to *C. septempunctata*; Roy *et al.* (2008), in their results about the adverse effects of *B. bassiana* towards three species of ladybird beetles, *Harmonia axyridis*, *C. septempunctata* and *Adalia bipunctata*, mentioned the negative effects towards these predators.

In case of aphid treatments, the present results indicated that the cabbage aphid was highly sensitive towards the two entomopathogenic fungi. This results were in accordance with those obtained by Vandenberg (1996) and Hatting and Wraight (2004), on controlling Russian wheat aphid (Homoptera: Aphididae); Nielsen and Hajek (2005) and Koch and Ragsdale (2007) for controlling of invasive soybean aphid, *Aphis glycines*.

In case of Bioranza, all tested concentration induced considerable percentages of mortality. At 4.0 and 2.0g/L concentrations, the percentage of mortality was 100 and 82%, respectively. While it was 49 and 28%, at 1.0 and 0.5g/L, respectively. Statistically, all tested concentrations exhibited highly significant difference among each other (P>0.01) (Fig. 2).

In case of Biovar treatment, the lowest tested concentration (0.5g/L) induced the lowest percent

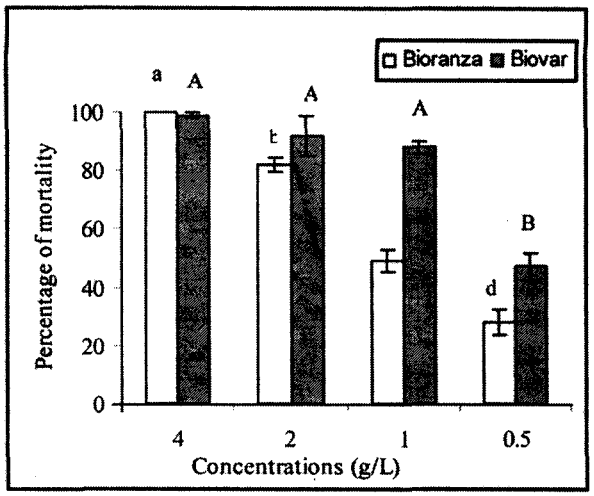

Fig. (3): *Brevicoryne brassicae* corrected percentage of mortality as a result of treatment with the fungal formulations at different concentrations.

mortality (47.00%), being significantly different from the other tested concentrations ($P>0.01$). The other tested concentrations (4.0, 2.0 and 1.0g/L) induced 99.00, 92.00 and 88.00% mortality, different insignificantly from each other ($P<0.05$) (Fig. 3).

Statistically, there were no significant differences between the two fungi formulations at 4.0 and 2.0g/L ($P<0.05$), while significant differences were observed between the two other concentrations (1.0 and 0.5g/L) (Fig. 3).

The LC_{50} value for Bioranza was 0.890g/L, while it was 0.450g/L in case of Biovar. It was noticed that the LC_{50} value of Bioranza was about twice its value in case of Biovar (Table 1), *i.e.*, Biovar formulation was still more efficient than Bioranza.

The LT_{50} values of tested concentrations indicated that, at all tested concentrations, Biovar formulation was faster than Biovar. These results were in accordance with those reported by Chandler (1997) for evaluating of *M. anisopliae* virulent against the lettuce root aphid, *Pemphigus bursarius*.; Milner (2000); Olson *et al.*, (2002), who reported that the entomopathogenic fungi attacks wide range of insects including beneficial and harm species and the susceptibility varied according to the species and concentration used; Filotas *et al.* (2004) and Ugine *et al.* (2007), gave a reports about the effects of the fungi formulations against the pests and the beneficial insects.

From the aforementioned results, it could be concluded that, the use of either formulations at a concentration of 1.0 or 0.05g/L can induce a satisfied considerable percent mortality, under greenhouses or open fields, among the cabbage aphid, *B. brassicae* population with low or minimal damage to its predator, *C. undecimpunctata* population.

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