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Efficacy of *Beauveria bassiana* (Deuteromycotina: Hypomycetes) Against Different Aphid Species Under Laboratory Conditions

Muhammad Akmal, Shoaib Freed*, Muhammad Naeem Malik and Hafiza Tahira Gul
Department of Agricultural Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Punjab, Pakistan.

Abstract.- In the recent study, the entomopathogenic fungus, *Beauveria bassiana* was used to evaluate its pathogenecity against adults of different aphid species *i.e., Schizaphis graminum, Rhopalosiphum padi*, *Brevicoryne brassicae* and *Lipaphis erysimi*, and their natural enemy *Coccinella septempunctata*. *B. bassiana* was found effective at all concentrations *i.e.*, 1×10^6, 1×10^7 and 1×10^8 spores/ml on all aphid species, but the uppermost concentration (1×10^8 spores/ml) provided maximum control within short period of time ($P < 0.0001$). *B. bassiana* with LC_{50} value of 6.28×10^5 proved to be lethal to *B. brassicae* after 3rd day. Mortality of *L. erysimi* was observed maximum on highest concentration of 1×10^8 spores/ml with LC_{50} value of 1.36×10^6, while the LT_{50} values were in range of 2.19-3.73 days for different aphid species treated at various concentrations. *B. bassiana* showed little or no detrimental effects to *C. septempunctata*. By testing the field efficacy of *B. bassiana* against aphid species, this insect pathogenic fungus can be used as potential biocontrol agent for the management of aphids.

Keywords: *Beauveria bassiana*, mycosis, aphid, ladybird beetle, *Brassica*, entomopathogenic fungi.

INTRODUCTION

Aphids are serious pests of cultivated crops all over the world. Approximately, 92 species of aphids have been recorded in Pakistan (Irshad, 2001). On cereal crops, aphid population has been increasing continuously for the last few years and attaining the pest status in Pakistan (Zia *et al.*, 1999). The wheat aphids, *Schizaphis graminum* and *Rhopalosiphum padi* cause severe harm to wheat crop throughout the world including Pakistan (Hamid, 1983; Inayatullah *et al.*, 1993), while *Brevicoryne brassicae* L. and *Lipaphis erysimi* (Kalt.) are destructive insect pests of *Brassica napus* in different districts of Southern Punjab, Pakistan, depending upon the severity of the invasion and crop stage (Rana, 2005).

The sap sucking aphids may cause great damage directly by nutrient exhaust or indirectly by more than a few mechanisms, including virus transmission. Due to parthenogenetic reproduction and short generation times, aphid population increases rapidly (Hales *et al*., 1997) that can reach the pest status. Management of aphids has been done primarily by using chemical methods; however environmental and health problems have arisen due to this practice (Botto, 1999).

Widespread use of insecure synthetic chemical pesticides and demanding crop production causes several socio-economic problems throughout the world. For example, more than twenty aphid species together with *Myzus persicae* (Foster *et al*., 1997; Harrington and Emden, 2007) have showed resistance to a number of carbamate, pyrethroid and organophosphate based insecticides. Entomo-pathogenic fungi provide an environmentally responsive substitute to chemical pesticides. They are natural, easy to formulate, less toxic to mammals, with no residual activity (Copping, 2004) and less chance to develop resistance (Zimmermann, 2007). *Beauveria bassiana* and *Metarhizium anisopliae* have a wide host range (Butt *et al*., 1994), widely distributed in all regions of the world and can be easily isolated from insects, soil and phylloplanes of vegetation (Meyling *et al*., 2006; Freed *et al.*, 2011a,b).

Insect pathogenic fungi are a component within integrated pest management systems and have great capacity as biological control agents against insects. Hyphomycete fungi are inexpensive for mass production, easy to store and efficient over an extensive range of temperature and humidity. These also offer a swift eradication at cost-effective doses. For the control of a large number of

* Corresponding author: sfareed@bzu.edu.pk; shbfkh@gmail.com
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agricultural pests entomopathogenic fungi are being developed throughout the world (Ferron, 1985) and some are already available commercially for the control of various species of thrips and aphids (Goettel *et al*., 1990; Upadhyay, 2003).

Entomopathogenic fungi that attack insects are important agents for biocontrol and have a vital part in promoting integrated pest management (Cooke, 1977). Up till now, a variety of strains of entomopathogenic fungi such as *Lecanicillium* sp. (Jackson *et al.,* 1985, Jung *et al.*, 2006, Steenberg and Humber, 1999; Kim, 2004), *B. bassiana* (Quesada-Moraga *et al*., 2006), *M. anisopliae* (Shia and Feng, 2004; Wright *et al*., 2004), *Isaria* (Shia and Feng, 2004), and *Nomuraea rileyi* (Devi *et al*., 2003) have been used for the management of aphids and many other pests.

Previous investigations concerning the efficacy of diverse fungal isolates for the management of aphids have showed greater success (Lacey *et al*., 1997; Askary *et al*., 1998; Irshad, 2001; Vandenberg *et al*., 2001; Alavo *et al*., 2002; Hatting *et al*., 2004; Steinkraus and Boys, 2005; Aiuchi *et al*., 2007; Kim *et al*., 2007; Hesketh *et al*., 2008; Kim and Kim, 2008). Owing to the increasing threats of aphids in the region and indirect damage by the injudicious use of pesticides, a study was planned to check the efficacy of *B. bassiana* against different aphid species *viz., S. graminum, R. padi, B. brassicae, L. erysimi* and also to check its compatibility with the biological control agent of aphids *i.e., Coccinella septempunctata.*

MATERIALS AND METHODS

Four species of aphids *i.e., S. graminum, R. padi, B. brassicae* and *L. erysimi* were collected from wheat and *Brassica* fields. Different aphid species were placed in ventilated plastic jars (10 × 6cm) and *Brassica* leaves were used as food for aphids of *Brassica* crop and wheat leaves for aphids of wheat. After checking for disease and parasitism, healthy individuals were used in pathogenecity assays. Three different conidial suspensions *i.e.*, 1×10^6, 1×10^7 and 1×10^8 of entomopathogenic fungi *B. bassiana* (BB-01) were set from 14 days old fungus culture in 0.05% Tween 80 solution. For each treatment a 50mm diameter leaf disc was cut out of a healthy mustard plant and dipped into 5ml of conidial suspension for 10s while excess suspension was removed by placing the leaf discs on sterile filter paper for few minutes, while control leaf discs were treated with 0.05% Tween 80 only. These discs were then placed on moist filter paper in plastic petri plates. Twenty-five healthy aphids per replication were placed on treated and untreated leaf disc and incubated at 23±2°C with a 16:8 L: D. Same procedure was applied to the wheat aphids and wheat leaves were used instead of *Brassica*. There were four treatments including control and each treatment was replicated five times. The mortality data was recorded over a period of seven days. Cadavers were shifted to petri dishes with moist filter paper to promote fungal development and sporulation in order to confirm that death is due to fungal infection.

Toxicity of B. bassiana *against* Coccinella septempunctata

The natural control agent of aphids *i.e.,* ladybird beetle *C. septempunctata* were also treated with *B. bassiana*. For this purpose five adults of ladybird beetle per replication were treated with same concentrations of fungi and kept in petri plates under same conditions as described above. Alive aphids were provided daily as a food source. The beetles were monitored daily and the mortality data was recorded for consecutive seven days. There were four treatments including control and each treatment was replicated five times.

Data analysis

The experiment was designed under Completely Randomized Design with four treatments including control and each treatment was replicated five times. The means for the corrected mortality were separated by Duncan's Multiple Range Test (DMRT). All the statistical analysis was performed by using SAS (SAS, 2002), while probit analysis was used to calculate the LC_{50} and LT_{50} values.

RESULTS

Effect of B. bassiana *on* B. brassicae

The fungal treatment showed significant

mortality from the first day but maximum mortality of 100% was obtained at 7th day post treatment at a concentration of 1×10^8 ($F_{4,6}$ = 879.37; P < 0.0001), while minimum mortality of 99.2% was obtained with treatment of 1×10^6 ($F_{4,6}$ = 105.05; P < 0.0001) (Fig.1A). In contrast to this no mortality was recorded in control. The values of LC_{50}, 6.28×10^5 (1.80×10^1-2.19×10^8) (Table I) showed that the 50% mortality was obtained at 3rd day of treatment, while LT_{50} was in range of 2.38-2.86 at different concentrations, respectively (Table II).

Effect of B. bassiana *on* L. erysimi

The fungal application on *L. erysimi* showed effectiveness after 4th day when 50% of tested aphid population was killed but with the passage of time at 7th day 100% mortality was observed at all concentrations ($F_{4,6}$ = 691.91; P < 0.0001) (Fig. 1B) while no mortality was recorded in control. The values of LC_{50}, 1.36×10^6 (7.66×10^5-2.42×10^6) (Table I) showed that the 50% mortality was recorded at 4th day of treatment, while LT_{50} was in range of 2.70-3.73 at different concentrations, respectively (Table II).

Effect of B. bassiana *on* S. graminum

The effect of entomopathogenic fungus on *S. graminum* showed significant results from the 2nd day and more than 90% mortality was obtained at 6th day ($F_{4,6}$ = 221.74; P < 0.0001). The results showed that mortality was dose dependent which increased with enhancing the concentration (Fig.1C) while no mortality was recorded in control. The LC_{50} values 6.76×10^6 (2.84×10^6- 1.61×10^7) (Table I) showed that the 50% mortality was recorded at 3rd day post treatment, while LT_{50} was calculated in range of 2.79-3.69 at all tested concentrations (Table II).

Effect of B. bassiana *on* R. padi

The efficacy of *B. bassiana* was evaluated on *R. padi* which showed significant results at all concentrations, while maximum percent mortality of 98.40 was recorded after 7th day of treatment with a concentration of 1×10^8 ($F_{4,6}$ = 210.54; P < 0.0001); in contrast to this minimum percent mortality of 85.60 was obtained by the application of lowest concentration *i.e.*, 1×10^6 ($F_{4,6}$ = 77.99; P < 0.0001) (Fig.1D) while no mortality was recorded in control. The LC_{50} values 1.15×10^6 (3.68×10^5- 3.59×10^6) (Table 1) showed that 50% mortality was recorded at 4th day post treatment, while LT_{50} was calculated in range of 2.19-3.20 at different concentrations, respectively (Table II).

Effect of B. bassiana *on* C. septempunctata

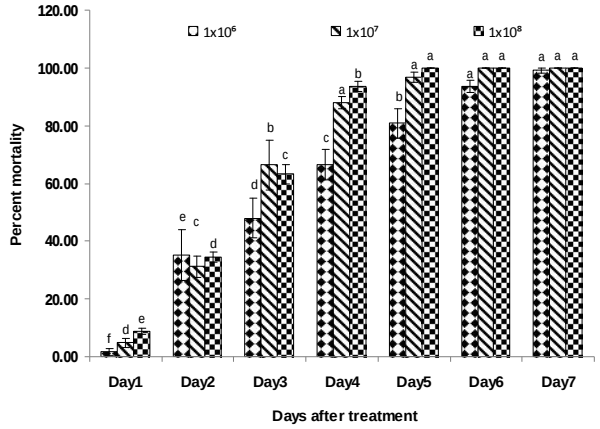
The natural enemy of aphids was checked for its compatibility with entomopathogenic fungi. The application of *B. bassiana* showed little or no detrimental effects against *C. septempunctata*. The fungus proved to be beneficial for the lady birds as on 7th day post treatment 10% mortality was observed at maximum concentration of 1×10^8 (Fig.1E).

DISCUSSION

The current study shows the effectiveness of *B. bassiana* against different aphid species. The study showed that *B. bassiana* is effective for the control of aphids on different crops at different concentrations. The fungi causing pathogenecity in insects have been observed to cause mortality in insect pest populations and therefore studied for their use as biological control agents (Butt *et al.*, 1994, 1995; Hesketh *et al.*, 2008; Freed *et al.*, 2012) or effectively developed for the biological control of several insect pests, which include aphids also (Shah and Pell, 2003; De Faria and Wraight, 2007).

The entomopathogenic fungi virulence potential against target aphid populations is different for different isolates and also varies from strain to strain. The similar aphid specie can have different susceptibility ratio to different fungal strains. The susceptibility of biotypes of the same aphid species may vary for fungal infectivity (Blanford *et al.*, 2003; Ferrari *et al.*, 2001). Some soil derived isolates of *B. bassiana* resulted in higher mortality when compared to other isolates of coleoptera and orthoptera (Goettel *et al.*, 1990).

Different concentrations of fungal isolates were tested against the adults of aphids. The concentration of conidia affected the mortality of aphids differently (P<0.0001). The aphid mortality was recorded at 24 h interval for consecutive seven days. Mortality of aphids on the first day did not



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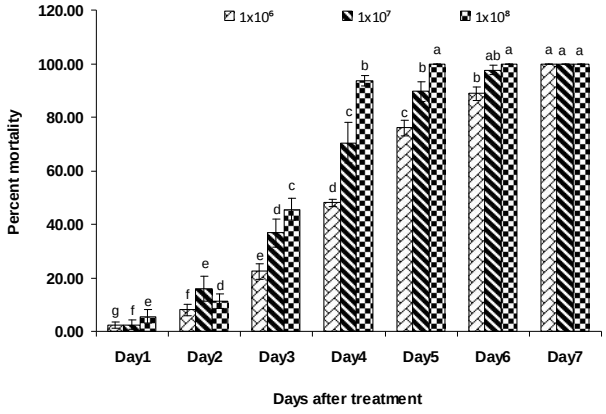

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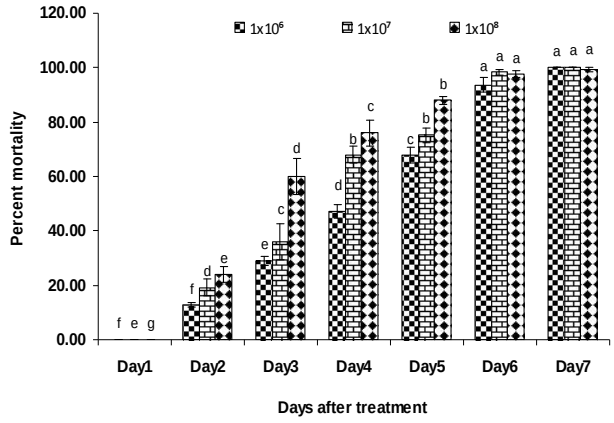

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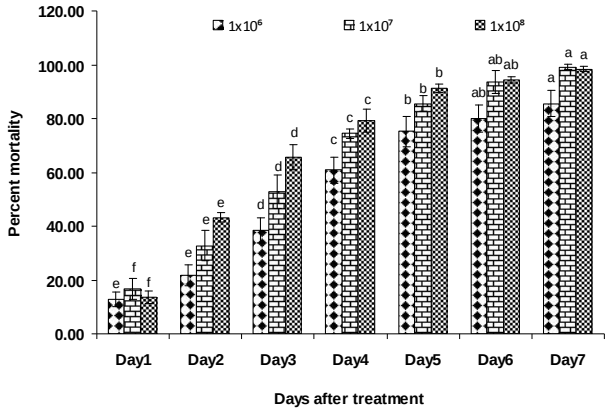

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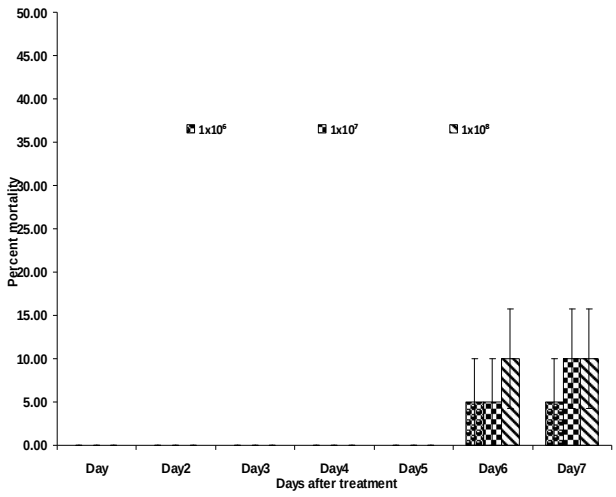

E

Fig. 1. Mortality of adult aphids *B. brassicae* (A) L. erysimi (B), *S. graminum* (C) *R. padi* (D), *C. septempunctata* (E) after exposure to *B. bassiana* (BB-01). For each day the same letters are not significantly different ($P < 0.0001$) according to Duncan's Multiple Range Test (DMRT).

Table I.- LC_{50} of *B. bassiana* (BB-01) on different days against various aphid species.

Aphid	Day	LC_{50}	FD Limit	Slope	DF
B. brassicae	3rd	6.28×10^{5}	$(1.80\times10^{1}$-$2.19\times10^{8})$	0.20±0.16	1
L. erysimi	4th	1.36×10^{6}	$(7.66\times10^{5}$-$2.42\times10^{6})$	0.75±0.10	1
S. graminum	3rd	6.76×10^{6}	$(2.84\times10^{6}$-$1.61\times10^{7})$	0.35±0.08	1
R. padi	4th	1.15×10^{6}	$(3.68\times10^{5}$-$3.59\times10^{6})$	0.39±0.08	1

Table II.- LT_{50} of different concentrations of *B. bassiana* (BB-01) against various aphid species.

Aphid	Concentration	LT_{50}	FD Limit	Slope	DF
B. brassicae	1×10^{6}	2.86	(2.30-3.56)	4.23±0.48	5
	1×10^{7}	2.54	(0.83-7.78)	3.25±0.78	5
	1×10^{8}	2.38	(1.31-4.32)	3.72±0.78	5
L. erysimi	1×10^{6}	3.73	(1.54-9.03)	5.50±1.48	5
	1×10^{7}	3.08	(2.28-4.16)	5.63±0.83	5
	1×10^{8}	2.70	(0.37-19.77)	6.13±2.74	5
S.graminum	1×10^{6}	3.69	(2.81-4.86)	5.59±0.84	5
	1×10^{7}	3.24	(2.49-4.21)	5.48±0.76	5
	1×10^{8}	2.79	(2.65-2.95)	5.41±0.32	5
R. padi	1×10^{6}	3.20	(2.63-3.90)	2.88±0.32	5
	1×10^{7}	2.45	(1.90-3.17)	3.14±0.40	5
	1×10^{8}	2.19	(2.03-2.37)	3.56±0.21	5

differ significantly from the control group. It can be owing to the development of infection in insect tissues. *B. brassicae* at different time intervals indicates that the mortality increased with increase in time interval (Fig. 1A) with LC_{50} value of 6.28×10^{5} after 3rd day (Table I). In contrast to this the mortality of *L. erysimi* was observed maximum on highest concentration of 1×10^{8} spores mL^{-1} of *B. bassiana* while minimum on lower doses of 1×10^{6} and 1×10^{7} spores mL^{-1} (Fig. 1B) with LC_{50} value of 1.36×10^{6}. The results described herein are in accordance with Halimona and Jankevica (2011) in which different entomopathogenic fungi were used against *Aphis fabae* and *Metopeurum fuscoviride*. Similar results were obtained when individuals of *S. graminum* and *R. padi* were exposed to the all three concentrations of *B. bassiana* and the highest concentration *i.e.*, 1×10^{8} spores mL^{-1} showed the maximum percent mortality on the seventh day of fungal treatment (Figs. 1C,D).

Time-dose dependent mortality response was checked on different aphid species after application of the pathogenic fungus. The mortality observed was low on day 1-2 after treatment in all fungal isolates, it increased gradually and maximum mortality was obtained on day 4-7 and minimum values of LT_{50} were 2.19 days for *R. padi* and maximum value of 2.79 days was recorded for *S. graminum* (Table II). The mortality in infected aphids with *B. bassiana* increased with increase in spore concentration of conidial suspensions and exposure time. The susceptibility of target insect to fungal infection is dose dependent (Liu *et al.*, 2002; Wright *et al.*, 2005). Ansari *et al.*, (2004) also found that mortality depended on the concentration of conidial suspension, exposure time and temperature. The susceptibility of same aphid species may vary to different fungal strains. Even biotypes or different colons of the same aphid species may have changeable susceptibility to fungal contagion (Ferrari *et al*., 2001; Blanford *et al*., 2003). It is economically important to determine the optimal concentration of conidia for spray applications to reduce the overall cost of pest control while achieving high control efficiency. The results (Figs. 1A-D, Tables I, II) illustrate that mortality caused

by *B. bassiana* by using the concentration of 1×10^8 conidia ml^{-1} was significantly higher ($P<0.0001$) than those of 1×10^6 and 1×10^7 conidia ml^{-1}. Therefore concentration of 1×10^8 conidia ml^{-1} can be used to control aphids (Vue *et al*., 2007).

Another aspect of current study was to check the effect of *B. bassiana* on aphid predator *C. septempunctata*. The adult *C. septempunctata* were exposed to all three different doses of *B. bassiana* used against aphid. *C. septempunctata* showed resistance against *B. bassiana* and no mortality was observed upto 5 days post treatment. Only five percent mortality was recorded on the 7^{th} day of treatment at lower dose (1×10^6) and maximum 10% mortality was observed on higher doses of 1×10^7 and 1×10^8 spores ml^{-1} (Fig. 1E). The results are in accordance to that of Thungrabeab and Tongma (2007), in which *C. septempunctata* showed resistance against *B. bassiana* and no mortality was observed. While in contrary to this the results reported herein are not in harmony to the findings of James and Lighthart (1994) who reported the potential of *M. anisopliae*, *B. bassiana* and *Paecilomyces fumosoroseus* to infect *Hippodamia convergens* Guérin Méneville (Coleoptera: Coccinellidae), while *Nomuraea rileyi* was found safe for the beneficial.

From our results it is concluded that *B. bassiana* is effective at all concentrations of 1×10^6, 1×10^7 and 1×10^8 spores/ml on the four species of aphid *B. brassicae*, *L. erysimi*, *S. graminum* and *R. padi*, but the highest concentration (1×10^8 spores/ml) can give maximum control within short period of time. However its field application is needed to be done to confirm the results.

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