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EFFICACY OF *Beauveria bassiana* AND *Verticillium lecanii* FOR THE MANAGEMENT OF WHITEFLY AND APHID

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In the present study two entomopathogenic fungi, *Beauveria bassiana* and *Verticillium lecanii*, were tested for their efficacy against whitefly and aphid. The mortality caused by *B. bassiana* was higher as compared to *V. lecanii*. The mortalities caused by filtrates were significantly higher than those by conidia of both the fungi. In case of whitefly, the mortality caused by *B. bassiana* was significantly higher than that of *V. lecanii*. Similarly, the mortality with filtrates was significantly greater than that with conidia of both the fungi. On the other hand, in case of aphid, the mortality caused by *V. lecanii* was slightly higher than that by *B. bassiana*. Similar is the case with formulations. The mortality caused by conidia was greater than by filtrates of both the fungi but the difference was non-significant. As regards treatments, the maximum mortality of whitefly was obtained with treatment *B. bassiana* Bb01 followed by *V. lecanii* Vl01 and *B. bassiana* Bb02 when applied as filtrates. The treatment *V. lecanii* V3 caused the minimum mortality followed by *V. lecanii* V2. The filtrate formulations caused higher mortalities as compared to conidial application. On the contrary, the treatments in case of aphid mortality were at par with each other with few exceptions. Maximum aphid mortality was observed with treatment *V. lecanii* V2 followed by *B. bassiana* Bb02. The remaining treatments almost gave the similar mortalities. Time intervals also affected mortality. The mortality was significantly greater after exposure to 6 days as compared to 3 days.

Keywords: Insect pests, Aleyrodidae, entomopathogens, phytopathogens, biocontrol, virulence.

INTRODUCTION

A large number of biotic factors are responsible for incurring yield losses in crops, fruits and vegetables around the globe and in Pakistan. These include insect pests of different kinds which affect the crops qualitatively and quantitatively (Iftikhar *et al*., 2018; Javed *et al*., 2017a,b; Kassi *et al*., 2018, 2019a,b; Nabeel *et al*., 2018; Aslam *et al*., 2019a). Among phytopathogens, viruses (Ashfaq *et al*., 2017), fungi (Butt *et al*., 2016; Fateh *et al*., 2017), nematodes (Hussain *et al*., 2016; Kayani *et al*., 2017, 2018; Khan *et al*., 2017; Tariq-Khan *et al*., 2017; Mukhtar *et al*., 2017a,b, 2018; Hussain and Mukhtar, 2019), and bacteria (Aslam *et al*., 2017a,b, 2019b) also cause severe growth retardations.

Whitefly (*Bemisia tabaci* Genn.) belonging to the order Hemiptera and family Aleyrodidae is considered as a serious threat to agricultural crops. It is a wide spread pest worldwide and known to transmit many viruses in field crops. There are many biotypes of whitefly reported as economic pests throughout the world. A well-known biotype B of *B. tabaci* is a major pest of many crops and vegetables causing losses worth 714 million dollars per year (Oliveira *et al*., 2013). Aphid is also a devastating pest of a wide range of crops grown in greenhouses like pepper, tomato and cucumber. *Sitobion avenae, Rophalosiphum padi, Schizaphis graminum, Metopolophium dirhodum* are reported species of aphid causing serious damage to field crops (Dana *et al*., 2006). Aphid and whitefly rapidly increase their populations and are mostly found in overlapping generations. Insecticides are used as primary tactic to control these pests in greenhouses and field crops (Castle *et al*., 2014) but the increasing use of insecticides at higher doses has resulted in resistance, resurgence and high input costs. The environmental and legal restrictions due to the use of higher doses of insecticides have urged the search for alternative control methods including biological control in general and use of entomopathogens in specific (Kayani and Mukhtar, 2018; Mukhtar, 2018; Rahoo *et al*., 2018a,b).

Entomopathogenic fungi have the exclusive ability to be used as biological control agents as they can infect their host directly through the integument. These fungi are widely spread in the world and adapted to terrestrial agro-ecosystems where insects or other arthropods are available. Many important insect pests belonging to insect orders Coleoptera, Lepidoptera, Isoptera and Hemiptera have been reported to be susceptible to many isolates of entomopathogenic fungi and nematodes (Boopathi *et al*., 2015; Rahoo *et al*., 2017, 2019a,b). Natural mortality in whitefly population has been reported due to entomopathogenic fungi mostly by *Aschersonia* spp., *Beauveria bassiana*, *Lecanicillium* spp. and *Isaria fumosorosea* (Ascomycota: Hypocreales) (Wraight *et al*., 2007).

Different isolates of *Verticillium lecanii*, *B. bassiana*, *Paecilomyces lilacinus* and *Metarhizium anisopliae* have been reported to be used for the control of many insect pests in China. Among these, *B. bassiana* is the most spread and easily available species (Aung *et al*., 2008). Whitefly and aphid were best controlled by *B. bassiana* (Bb04) while minimum mortality was achieved by strain of *B. bassiana*. It was also reported that *B. bassiana* is only restricted to the natural habitat while another species *M. anisopliae* is manageable in agro-ecosystem because it can persist in cultivable soils also (Sánchez-Peña *et al*., 2011). The control of whitefly and aphid was also reported by two different isolates of *V. lecanii* in Netherlands (Milner, 1997). Many species of entomopathogenic fungi have also been reported from Korea controlling a number of aphid species (Yoon *et al*., 1999).

As there is little information regarding virulence of different isolates and efficacy of entomopathogenic fungi against whitefly and aphid, therefore, the present study was focused to evaluate the efficacy of different isolates of two entomopathogenic fungi (*Beauveria bassiana* and *Verticillium lecanii*) under laboratory conditions as biological control agents against whitefly and aphid. The study will help to understand strategies towards alternatives to insecticidal control of these pests.

MATERIALS AND METHODS

Whitefly and aphid populations**:** Whitefly was reared on potted tomato seedlings following the method described by Borisade (2015). Adults of whitefly were released to these plants 14 days after transplantation. The potted tomato plants were covered with insect nets (mesh size = 0.0322×0.0105) to prevent the movement of insects across the pots. The plants were maintained under laboratory conditions of 24±2°C, 55±3% relative humidity and 16:8 (Light:Dark) photoperiod and egg laying was continuously observed (Samih *et al*., 2006). *Myzus persicae* (Sulzer) commonly known as green peach aphid was collected from cabbage leaves in greenhouse conditions at Chinese Academy of Agricultural Sciences, Beijing, China and shifted to bean plants already potted under insect nets maintained at 24°C, 45-60% relative humidity and 16:8 (Light:Dark) photoperiod in laboratory (Khan *et al*., 2012). The plants were replaced every week to provide enough nutrition for reared insects.

Fungal isolates**:** Three fungal isolates each of *B. bassiana* and *V. lecanii* given in Table 1 were evaluated against whitefly and aphid. The fungal isolates were maintained on Potato Dextrose Agar (PDA) slants in tubes at 4°C. The isolates were grown on PDA at 25°C for 14 days and stored at 4°C.

Table 1. Detail of entomopathogenic fungi used in the study.

Isolate	Isolate	Original Host	Geographical Region
Beauveria bassiana	Bb01	Gray weevil	Lahore (Pakistan)
Beauveria bassiana	Bb02	Gray weevil	Multan (Pakistan)
Beauveria bassiana	Bb03	Cotton bug	RYK (Pakistan)
Verticillium lecanii	Vl01	Moth	Lahore (Pakistan)
Verticillium lecanii 2	V2	Whitefly	Vladivostok (Russia)
Verticillium lecanii 3	V3	Whitefly	Moscow (Russia)

Conidial suspension**:** Conidial suspensions of different isolates of *B. bassiana* and *V. lecanii* were prepared by harvesting conidia from culture plates on the 20th day of culturing in 0.02% tween solution. The suspensions were vigorously stirred and filtered through sterile cheese cloth. Haemocytometer was used to count the conidia in suspensions under microscope and finally adjusted to 10^8 conidia per milliliter. The viability of conidia was checked following the method described by Hywell-Jones and Gillespie (1990).

Fungal filtrate**:** Filtrates of isolates of both the fungi were prepared by following the method described by Khan *et al*. (2012). Four milliliters of the conidial suspensions of all the isolates were poured separately in 100 ml of Adamek's Liquid Medium and incubated for three days in a shaking incubator at 150 rpm. For preparation of 1% secondary culture, 2.5 ml of primary culture was mixed with 250 ml of Adamek's Liquid Medium and incubated for six days at 26°C in a shaking incubator at 150 rpm. The solution was filtered, centrifuged at 10000 rpm at 4°C for half an hour and again filtered using Millipore corp of 0.45 µm sized pore.

Filtrate bioassay**:** Each treatment consisted of two plants attacked by aphids @ 70 to 90 aphids per plant. The plants were sprayed with 3 ml of filtrates from each treatment. The control was sprayed only by medium. Four leaves were cut from each treatment and placed in Petri dishes (one leaf in one Petri dish), stored at 24°C and 100% RH. A photoperiod of 16:8 (L: D) was maintained. Leaf dip method was used for whiteflies maintaining 20 whiteflies per Petri dish. The concentration and incubation period were the same as for aphid. Mortality was recorded on daily basis for all fungal isolates.

Conidial bioassay**:** Virulence of fungal isolates against whitefly was observed after spraying the conidial suspension in Petri plates having 20 adult whiteflies in each. The Petri plates were put into a Petri dish having 1% agar to help conidial germination by maintaining at 100% RH. Virulence of fungal isolates against aphids was detected after conidial shower on cabbage leaves placed in Petri plates and attacked by aphid @ 40-50 aphids per leaf. The Petri plates were put into a Petri dish having 1% agar to help conidial germination by maintaining 100% RH. The spores' concentration was maintained at 150 to 200 spores per millimeter square and counting was done through fields of cover slips placed in Petri dishes. The whitefly and aphid mortalities were observed after every 24 hours for 6 days continuously. The dead aphids and whiteflies were collected from Petri plates and preserved in dark at 100% RH. The individuals having fungal growth on their bodies after several days of storage were considered dead. Untreated leaves were used as control in aphids and untreated Petri plates were considered as control for whiteflies. There were 3 replications for all the isolates.

***Data analysis*:** The data were analyzed by one-way analysis of variance (ANOVA) using completely randomized design. The data were statistically analyzed by using software Statistix (8.1).

RESULTS

Both the entomopathogenic fungi caused mortalities of both the insect pests. The mortality caused by *Beauveria bassiana* (46.33%) was higher as compared to *Verticillium lecanii* (41.47%). The mortalities caused by filtrates were significantly higher than those by conidia of both the fungi. In case of whitefly, the mortality caused by *B. bassiana* was significantly higher than that of *V. lecanii*. Similarly, the mortality with filtrates was significantly greater than that with conidia of both the fungi (Fig. 1).

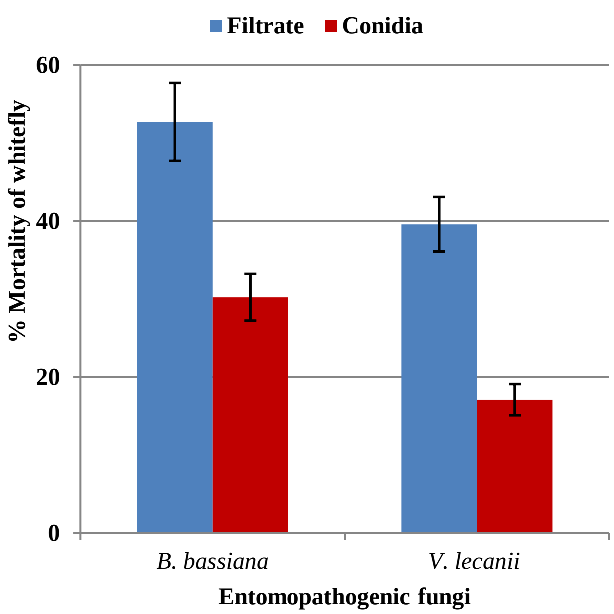

Figure 1. Effect of formulations of entomopathogenic fungi on percent mortality of whitefly.

On the other hand, in case of aphid, the mortality caused by *V. lecanii* was slightly higher than that by *B. bassiana*. Similar is the case with formulations. The mortality caused by conidia was greater than by filtrate of both the fungi but the difference was non-significant (Fig. 2).

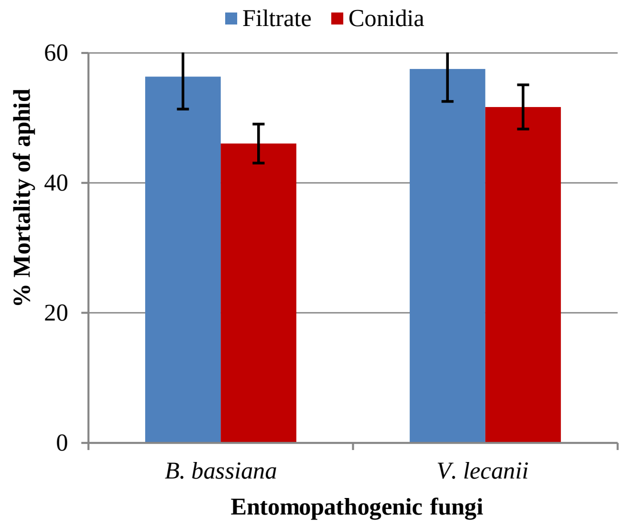

Figure 2. Effect of formulations of entomopathogenic fungi on percent mortality of aphid.

As regards treatments, the maximum mortality of whitefly was obtained with treatment *B. bassiana* Bb01 followed by *V. lecanii* Vl01 and *B. bassiana* Bb02 when applied as filtrates. The treatment *V. lecanii* V3 caused the minimum mortality followed by *V. lecanii* V2. The filtrate formulations caused higher mortalities as compared to conidial application (Fig. 3).

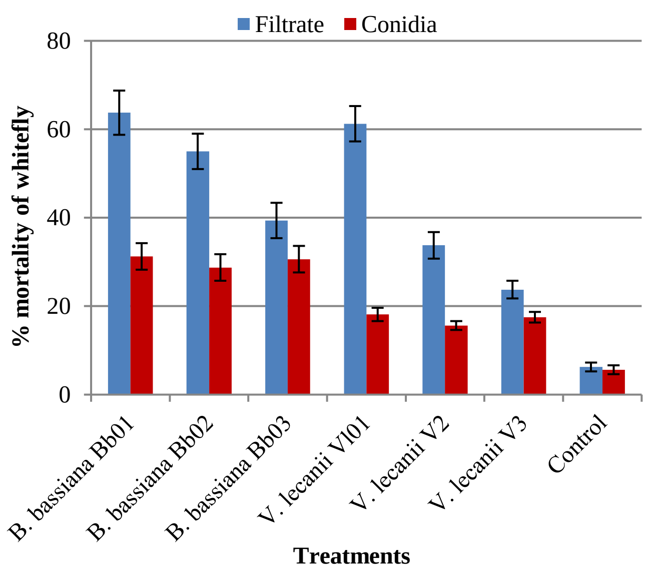

Figure 3. Effect of entomopathogenic fungi and their formulations on percent mortality of whitefly.

Time intervals also affected whitefly mortality. The mortality was significantly greater after exposure to 6 days as compared to 3 days. The individual mortalities of whitefly at both intervals are given in Table 2.

Table 2. Effect of entomopathogenic fungi on percent mortality of whitefly.

Treatment	% Mortality of whitefly in			
	Filtrate after		Conidia after	
	3 days	6 days	3 days	6 days
B. bassiana Bb01	38.75a	88.75a	22.50a	40.00ab
B. bassiana Bb02	31.25ab	78.75ab	8.75b	48.75a
B. bassiana Bb03	26.25abc	52.50abc	17.50a	43.75a
V. lecanii Vl01	33.75ab	88.75a	8.75b	27.50bc
V. lecanii V2	21.25abc	46.25bcd	7.50b	23.75cd
V. lecanii V3	8.75bc	38.75cd	8.75b	26.25bc
Control	3.75c	8.75d	2.50b	8.75d

On the contrary, the treatments in case of aphid mortality were at par with each other with few exceptions. Maximum aphid mortality was observed with treatment *V. lecanii* V2 followed by *B. bassiana* Bb02. The remaining treatments almost gave the similar mortalities. With few exceptions, no statistical significant differences were observed between mortalities with both the formulations as shown in Figure 4.

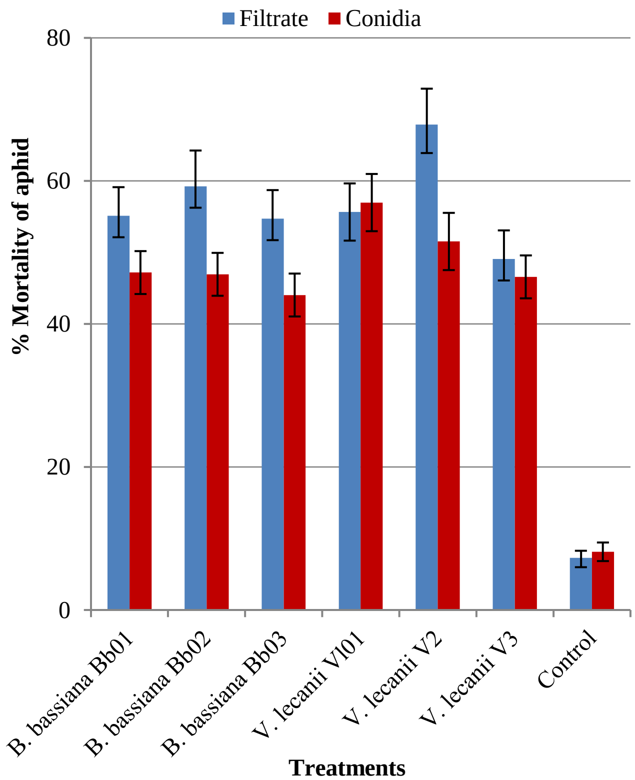

Figure 4. Effect of entomopathogenic fungi and their formulations on percent mortality of aphid.

Time durations also had significant effects on aphid mortality. After 6 days, the mortalities were significantly higher than those observed after 3 days. The mean individual mortalities of aphid obtained after both the intervals are shown in Table 3.

Table 3. Effect of entomopathogenic fungi on percent mortality of aphid.

Treatment	% Mortality of aphid in			
	Filtrate after		Conidia after	
	3 days	6 days	3 days	6 days
B. bassiana Bb01	35.00b	75.25ab	27.50c	66.87ab
B. bassiana Bb02	37.50b	81.00ab	38.07b	55.81b
B. bassiana Bb03	40.55ab	68.88bc	28.57c	59.52b
V. lecanii Vl01	39.28b	72.02abc	47.39a	66.55ab
V. lecanii V2	50.00a	85.79a	22.96c	80.10a
V. lecanii V3	39.02b	59.14c	25.56c	67.61ab
Control	4.16c	10.41d	7.95d	8.33c

DISCUSSION

The current study was focused on the toxicity or virulence of six different isolates of two entomopathogenic fungi with two different ways of application i.e. filtrate application and conidial spray. The filtrate application has shown highest mortality of whitefly and aphid. Kim *et al*. (2009) demonstrated that aphicidal activity was proportional to the concentration of the filtrate when compared with control. Khan *et al*. (2012) proved the same results showing 80% mortality by filtrate application of *B. bassiana* while 57% mortality was caused by conidia on the 6th day of application. The reason of low mortality by conidial application might be due to the more time required for conidial germination on insect body as compared with filtrate. Some other factors like viability of conidia, germination speed, growth rate for hyphae and effect of environmental factors (temperature, humidity, UV light) on spore production also influence the virulence of fungal isolates and their mode of action on different insects (Milner, 1997). Altre and Vandenberg (2001) also revealed in their studies that short life cycled insects are most susceptible to the filtrate-based application of entomopathogenic isolates as compared with the conidial application of the same isolates. The possible reason might be the less connectivity of conidia to insect bodies as compared to high penetration of filtrates. The present results of low mortality of whiteflies and aphids in conidial applications as compared with the filtrate applications are in line with the above mentioned results.

***Conclusion*:** The study revealed that the isolates of both the entomopathogenic fungi (*Beauveria bassiana* and *Verticillium lecanii*) have the potential to cause mortalities of whiteflies and aphids. Overall performance of all the fungal isolates was better in filtrate application rather than conidial application. *B. bassiana* (Bb01) was found to be the most virulent against whitefly while *Verticillium lecanii* (V2) appeared to be the most virulent against aphids after 6^{th} day of filtrate application. The introduction of these pathogenic fungi as biological control agents of these pests will save crops from indiscriminate use of insecticides. Further studies are required to standardize concentrations of these fungal isolates at different stages of these pests under field conditions.

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