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Effect of Conidial Concentration of Entomopathogenic Fungi on Mortality of Cabbage Aphid, *Brevicoryne brassicae* L.

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Abstract

An experiment was conducted to study effect of different conidial concentrations of some isolates of entomopathogenic fungi; *Verticillium lecanii* (V17), *Paecilomyces fumosoroseus* (n32), *Metarhizium anisopliae* (M440) and (L6) against adults of aphid, *Brevicoryne brassicae* L. under laboratory conditions at department of Entomology, University of Agriculture, Faisalabad during spring 2008.The experiment was conducted under CRD with four replications of each treatment. The concentration of conidia affected mortality of the aphids differently ($P < 0.001$) in all investigated fungal isolates. Mortality of the aphids increased with increase in spore concentration and exposure time. The fungal isolate *V. lecanii* (V17), with LC_{50} of 1.88×10^6 conidia ml^{-1} was considered the most virulent isolate among investigated fungal isolates followed by *P. fumosoroseus* (n32), *M. anisopliae* (L6) and *M. anisopliae* (M440) with LC_{50} values of 2.22×10^6, 3.48×10^6 and 5.30×10^6 conidia ml^{-1}, respectively 7 days after inoculation against the aphid.

Key Words: Conidial, concentration, entomopathologenic fungi, cabbage, aphid

Introduction

Insect pests are limiting factors for healthy growth of cultivated plants. Among insect pests, aphids are one of the most important arthropod pests of greenhouse and field crops through out the world. Their infestations cause severe economic losses as a result of crop yield reduction. Furthermore, aphids have been reported to be well known vectors of many viral diseases (Soomro *et al*., 1992: Emden and Harrington, 2007). Different strategies have been employed for control of this notorious pest. Farmers mostly prefer chemical pesticide application for its control because it is quicker one. However, due to adverse effects of insecticides on environment their rational use must be advocated.

Biological control is an alternative control method for insect pests. The biological control includes a very wide range of predators, parasitoids and pathogens. Naturally occurring entomopathogens are important regulatory factors in insect populations. Entomopathogenic fungi can serve as alternative to broad-spectrum chemical pesticides. When environmental benefits including safety for humans and other non target organisms, reduction of pesticide residues in food, increased activity of other natural enemies, and increased biodiversity in managed ecosystems are taken into consideration, their advantages over conventional insecticides are numerous. Unlike other microbial pathogens, such as bacteria, virus and protozoa, which infect the host via ingestion; entomopathogenic fungi invade the host by penetration of the outer integument. This enables parasitization of non-feeding, pupal and egg stages as well as the many sap and blood-sucking insects. Entomopathogenic fungi are found in the field under natural conditions, cause lethal infections and regulate insect populations in nature by epizootics.

Mitosporic entomopathogenic fungi in the order Hypocreales, such as *Beauveria bassiana*, *Lecanicillium longisporum* and *P. fumosoroseus* have been successfully developed as biological control agents against a number of different pests, including aphids (Faria and Wraight, 2007; Powell and Pell, 2007). Two isolates of *Verticillium lecanii* have been used successfully to control aphids on chrysanthemum and whitefly on cucumber and tomato (Milner 1997). Different products based on fungi against a variety of insects have been commercialized in different countries of world: e.g. Mycotal and Vertalec contain different strains of *V. lecanii*

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and are being used against homopteran in glasshouses. Using entomopathogenic fungi as mycoinsecticides against aphids has been successful (Shah and Pell, 2003; Faria and Wraight, 2007). Present study was conducted to evaluate the effect of conidial concentration of different fungal isolates on morality of aphid, *B. brassicae* L.

Materials and Methods

Fungus Culture

Fungal isolates; *P. fumosoroseus* (n32), *V. lecanii* (V17), *M. anisopliae* (M440) and (L6) used in this study were cultured on potato dextrose agar medium containing 20g glucose, 20g starch, 20g agar, and 1000 ml of distilled water in test tubes. The test tubes containing PDA medium were autoclaved at 121°C for 15-20 minutes and incubated at 25 ± 2°C, 80 ± 5% relative humidity and photo phase of 12 hours for 16 days after inoculation. The conidia were harvested by scraping the surface of 16-days old culture gently with inoculation needle. The conidia were suspended in distilled water containing 0.01% Tween-80. The mixture was stirred with a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using haemocytometer. Three droplets of a diluted suspension containing conidia at rate of 1 x 10^6 condia ml^{-1} were placed on potato dextrose agar medium and incubated at 25 ± 2°C and 80 ± 5% relative humidity in the dark for 24 hours. After staining with lacto-phenol cotton blue, germination was checked under microscope. Only spores with a germ tube as long as the conidium widths were considered to have germinated. In all tested isolates of entomopathogenic fungi more than 90% of the spores had germinated.

Rearing of Aphids

Cabbage plants were grown in earthen pots. All these potted plants were placed in wire house under semi-natural conditions. Aphids were procured from non infective area located in University of Agriculture Faisalabad. When plants were 22 days old then aphid were released on them. All agronomic practices were carried out in wire house under semi natural conditions. The aphid culture was maintained until all experiments were completed.

Bioassay procedure

Serial dilutions (1×10^3 to 1 x 10^7 conidia ml^{-1}) of each fungal isolate were prepared. Each concentration was considered one treatment. The detached leaf method was used for treatment of aphids with conidial concentrations. The cabbage leaves were sterilized with aqueous solution of sodium hypochlorite (0.5% v/v), washed three times with distilled water and then air dried. The leaves were placed on 1.5 % agar in 90 x 20 mm^2 plastic Petri dishes. The 1.5 % agar was non-nutritive and just to supply water to the leaves to maintain relative humidity during the test. A batch of 30 adult aphids (two days old each) was settled on each leaf, one day before treatment.

After treating each leaf by spraying it with 20 ml of conidial concentration of each fungal isolate, the leaves were placed on 1.5 % agar in plastic Petri dishes. These Petri plates were placed in an incubator maintained at 25 ± 2 °C and 80 ± 5 % R.H and photo phase of 12 hours. The aphids of the control were treated with 0.01% Tween 80. The experiment was conducted under CRD with four replications of each treatment and mortality of aphids was recorded daily up to 7 days. Mortality was corrected by using Abbott's formula (Abbot, 1925). Final data were subjected to proibit analysis (Mini tab 15), analysis of variance and DMRT using statistical package MSTATC.

Results

V. lecanii (V17)

The results show that the concentration of conidia affected the mortality of aphids differently ($P < 0.001$). This fungal isolate with LC_{50} value of 1.88×10^6 conidia ml^{-1} (Table 1) was found to be the most virulent among investigated fungal isolates against the adults of aphids 7days after treatment. The mortality caused by *V. lecanii* (V17) was 0.00-5.00% on the 1st day of treatment. The mortality then dramatically increased on day 5-7. The cumulative mortality caused by *V. lecanii* (V17) at the end of the observation (day 7) was 17.05-91.52%. The mortality increased with increase in spore concentration and exposure time.

P. fumosoroseus (n32)

The concentration of conidia of entomopathogenic fungus *P. fumosoroseus* (n32) also affected the mortality of aphids differently ($P < 0.001$). The LC_{50} of this fungus against the adults of aphids 7days after conidial treatment was determined to be 2.22×10^6 conidia ml^{-1} (Table 1). This fungus resulted in mortality of 0.00-5.83% on the 1st day of treatment (Table 3). The mortality then dramatically increased on day 5-7.The cumulative mortality caused by the fungus after 7 days of treatment was 18.14-90.82%. The mortality increased with increase in spore concentration and exposure time.

***M. anisopliae* (L6)**

The concentration of conidia affected the mortality of aphids differently ($P < 0.001$). The LC_{50} of this fungus against the adults of aphids 7days after conidial treatment was determined to be 3.48×10^6 conidia ml^{-1} (Table 1). The mortality caused by this fungus was minimum (0.00-3.33%) on the 1st day of treatment (Table 4). It increased with increase in spore concentration and exposure time. The cumulative mortality caused by the fungus at the end of the observation was 15.52-73.19 %.

***M. anisopliae* (M440)**

The results (Table 5) indicate that the concentration of conidia of *M. anisopliae* (M440) also affected the mortality of the aphids differently ($P < 0.001$). This fungal isolate with LC_{50} value of 5.30×10^6 conidia ml^{-1} (Table 1) was least effective among investigated fungal isolates against the adults of aphids 7days after conidial treatment. Mortality was recorded initially 24 hours after conidial treatment and it increased with increase in spore concentration and exposure time The mortality caused by this fungus was 0.00-5.83% on the 1st day of treatment (Table 5). The cumulative mortality caused by the fungus 7 days after treatment was 8.66-81.11%.

Discussion

Different concentrations of fungal isolates were tested against the adults of aphids. The concentration of conidia affected the mortality of aphids differently ($P < 0.001$). The fungal isolate *V. lecanii* (V17), with LC_{50} of 1.88×10^6 conidia ml^{-1} was considered to be the most virulent isolate among investigated fungal isolates followed by *P. fumosoroseus* (n32), *M. anisopliae* (L6), and *M. anisopliae* (M440) with LC_{50} values of 2.22×10^6, 3.48×10^6 and 5.30×10^6 conidia ml^{-1}, respectively 7 days after inoculation against the aphid. These findings indicate that LC_{50} values of different fungal isolates against same species of aphid depend upon the fungal species or its strains. Previous studies demonstrated that conidia of *V. lecanii* were highly pathogenic against aphids (Fournier and Brodeur, 2000; Kim *et al*., 2001).

The LC_{50} of *V. lecanii* (VL10) against *Myzes persicae* was observed to be 1.65×10^6 conidia ml^{-1} after six days of treatment (Yokomi and Gottwald, 1988).Vu *et al*. (2007) also reported that *V. lecanii* (41185) with LC_{50} value of 6.55×10^5 conidia ml^{-1} and its other strains were more lethal to aphid than fungal isolates of *P. farinosus* and *M. anisopliae*.

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 1-2 after treatment in all fungal isolates, it increased gradually on day 3-4 after treatment. The mortality then dramatically increased on day 5-7.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 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 varying susceptibility to fungal infection (Blanford *et al*., 2003 and Ferrari *et al*., 2001).

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 (Table3-5) show that mortality caused by concentration of 1×10^7 conidia ml^{-1} of all investigated fungal isolates was significantly higher ($P < 0.001$) than those of 1×10^5, 1×10^4, and 1×10^3 conidia ml^{-1}. Therefore concentration of 1×10^7 conidia ml^{-1} is the recommended concentration to spray to control aphids (Vue *et al*., 2007).

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Table 1 LC_{50} values of fungal isolates against cabbage aphid, *B. brassicae* L.

Isolate	LC_{50}	LFL	UFL
V.lecanii(V17)	1.88×10^6	1.32×10^5	2.50×10^6
P.fumosoroseus(n32)	2.22×10^6	1.64×10^6	2.85×10^6
M.anisopliae (L6)	3.48×10^6	2.77×10^6	4.32×10^6
M.anisopliae(M440)	5.30×10^6	4.50×10^6	6.15×10^6

Table 2 Effect of conidial concentration of *V. lecanii* (V17) against cabbage aphid, *B. brassicae* L.

Treatments (conidia ml^{-1})	Mortality (%) at indicated days after conidial treatments						
	1	2	3	4	5	6	7
$1x10^3$	0.0b	4.16c	6.67d	10.09d	11.82e	15.38d	17.05d
$1x10^4$	1.66b	5.00c	11.77cd	16.79c	22.07d	23.97d	24.21d
$1x10^5$	2.5ab	6.67bc	13.23c	22.04c	36.46c	39.02c	41.06c
$1x10^6$	2.5ab	10.73ab	20.78b	44.14b	53.90b	66.72b	75.04b
$1x10^7$	5.00a	13.28a	30.00a	54.22a	67.87a	88.16a	91.52a

Means with same letter are not significantly different from each other according to Duncan's Multiple Range Test at $P = 0.05$

Table 3 Effect of conidial concentration of *P. fumosoroseus* (n32) against cabbage aphid, *B. brassicae* L.

Treatments (conidia ml^{-1})	Mortality (%) at indicated days after conidial treatments						
	1	2	3	4	5	6	7
$1x10^3$	0.0b	3.33c	6.67d	8.48d	10.20d	11.98d	18.14d
$1x10^4$	0.0b	5.00bc	10.83cd	11.86d	15.23d	18.82d	21.63d
$1x10^5$	0.83b	7.50bc	13.33c	20.37c	26.29c	32.50c	34.56c
$1x10^6$	0.83b	9.17b	23.33b	35.66b	50.89b	59.41b	69.81b
$1x10^7$	5.83a	24.17a	47.50a	58.16a	67.85a	80.45a	90.82a

Means with same letter are not significantly different from each other according to Duncan's Multiple Range Test at $P = 0.05$

Table 4 Effect of conidial concentration of *M. anisopliae* (L6 against cabbage aphid, *B. brassicae* L.

Treatments (conidia ml^{-1})	Mortality (%) at indicated days after conidial treatments						
	1	2	3	4	5	6	7
$1x10^3$	0.0b	3.33c	6.67c	9.23d	11.74d	14.49d	15.52c
$1x10^4$	0.0b	6.67bc	10.00c	13.46c	16.07cd	16.24d	18.95c
$1x10^5$	0.0b	8.33b	10.83c	13.46c	18.62c	21.32c	27.63c
$1x10^6$	0.0b	9.95b	16.62b	29.39b	40.75b	53.02b	59.49b
$1x10^7$	3.33a	16.62a	26.64a	39.92a	54.19a	66.73a	73.19a

Means with same letter are not significantly different from each other according to Duncan's Multiple Range Test at $P = 0.05$

Table 5 Effect of conidial concentration of *M. anisopliae* (M440) against cabbage aphid, *B. brassicae* L.

Treatments (conidia ml^{-1})	Mortality (%) at indicated days after conidial treatments						
	1	2	3	4	5	6	7
$1x10^3$	0.0b	3.33c	5.00c	5.99c	6.83c	8.57c	8.66d
$1x10^4$	0.0b	4.16c	7.50c	8.50c	8.50c	11.99c	12.08cd
$1x10^5$	0.83b	5.00c	8.33bc	9.28c	12.68c	14.49c	15.50c
$1x10^6$	0.83b	10.73b	13.23b	19.51b	26.35b	28.22b	32.84b
$1x10^7$	5.83a	20.83a	32.50a	49.31a	66.17a	72.64a	81.11a

Means with same letter are not significantly different from each other according to Duncan's Multiple Range Test at $P = 0.05$