Pak. J. Bot., 44(6): 2081-2086, 2012.

USE OF ENTOMOPATHOGENIC FUNGI FOR THE CONTROL OF MUSTARD APHID (*LIPAPHIS ERYSIMI*) ON CANOLA (*BRASSICA NAPUS* L.)

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Abstract

The study was carried out to assess the virulence of 4 local and 3 exotic entomopathogenic fungal strains against mustard aphid (*Lipaphis erysimi* Kalt.) under laboratory and screen house conditions. According to LC_{50} and LT_{50} values, four strains *viz.*, *Lecanicillium lecanii* (PDRL922), *Paecilomyces lilacinus* (PDRL812), *Beauveria bassiana* (PDRL1187) and *Metarhizium anisopliae* (PDRL526) are found to be effective against the mustard aphid. The strain PDRL711 of *M. anisopliae* was found less effective, whereas, strains PDRL738 of *M. anisopliae* and PDRL1029 of *L. lecanii* were found avirulent. Based on the lab bioassays, each entomopathogenic fungal strain was used @ 10^7 c.f.u. ml^{-1} to evaluate its efficacy against mustard aphid infestation on canola plants under screen house conditions. Data showed a non-significant reduction in efficacy of different strains under screen house conditions as compared to lab bioassays. Strain PDRL922 reduced its efficacy from 98 to 83%, strain PDRL812 from 100 to 73% and Strain PDRL1187 from 88 to 77%. However, strain PDRL526 showed as increase in efficacy from 72 to 83% and strain PDRL711 from 44 to 70%. Strains PDRL1029 and PDRL738 appeared to be avirulent during lab as well as screen house trials. The study revealed that the strain PDRL526 of *M. anisopliae*, PDRL922 of *L. lecanii*, PDRL1187 of *B. bassiana*, PDRL812 of *P. lilacinus*, and PDRL711 of *M. anisopliae* have potential for use as bio-pesticides against mustard aphid under field conditions.

Introduction

Pakistan is one of the edible oil importing countries. The local production of edible oil is 0.680 million tons, which is almost 25% of total edible oil consumption. The rest has to be imported at the cost of Rs. 77.78 billions annually (Anon., 2010). Among the mustard family (Cruciferae), canola has wide acceptance as edible oil since it has lower concentration of erucic acid and glucosinolates (Love *et al.*, 1990). Canola has only 4.2% share in total edible oil production of Pakistan. Canola cultivation involves high input investment and low out puts in comparison to other winter crops. There are a number of limiting factors for more profitable outputs/production; insect pests are also one of the limiting factors for crop yield. Aphids are important insect pests of canola plant who suck the sap from plant, attract plant pathogen fungal spores by production of honey dew and also serve as a vector of plant viruses, ultimately playing a vital role in limiting the yield of the crop (Irshad, 2001; Emden & Harrington, 2007). Mustard aphid, *Lipaphis erysimi* Kalt., (Homoptera: Aphididae) has been reported as dominant pest over other insect pests on *Brassica* species (Farooq, 2007).

Use of Pesticide is common against aphids. Indiscriminate use of insecticides posed severe ecological changes, such as development of insecticide resistance in insect pests, adverse affects on friendly organisms, environmental pollution and accumulation of toxic elements in food and ultimately pesticide residue-induced diseases like cancer, kidney and liver failure, and genetical disorders in human beings (Lipson, 1997; Ambethger, 2009). Since small arthropod pests are known to easily develop resistance to chemical insecticides, interest in utilization of entomopathogenic fungi against them is increasing worldwide. Entomopathogenic fungi are important natural regulators of insect populations and have potential as bio-pesticide agents against diverse insect pests in agriculture (Steinhaus, 1958; Franz 1961; Laird, 1962; Hall, 1963; Fargues, 1975). Class Homoptera is important host of entomopathogenic fungi (Humber, 1989). There are several commercial myco-pesticide with different brand names and formulations which are easily available as agro-commodities world-wide (Faria & Wraight, 2007). The anamorphic entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are natural enemies of a wide range of insects and arachnids including aphids and mealy bugs; both fungi have a cosmopolitan distribution (Roberts & Leger, 2004; Rehner, 2005, Ujjan & Shahzad 2007). *Lecanicillium (Verticillium) lecanii* (Zimm.) Zare & Gams has been used against greenhouse whitefly, thrips and aphids (Khetan, 2001; Ambhethger, 2009; Asi *et al.*, 2009a&b). Similarly, *Paecilomyces fumosoroseus*, *P. farinosus* and *P. lilacinus* have been reported as entomopathogenic on a variety of insect pests (Meitkiewski *et al*., 1997; Pedro *et al*., 2001).

Very little information is available on the use of indigenous entomopathogenic fungi for the control of insect pests in Pakistan. Asi *et al*., (2009a,b) used a local strain of *M. anisopliae* against cabbage aphid *Brevicoryne barassicae* L. This strain has also been screened for its compatibility with insecticides (Asi *et al.*, 2010). Similarly, two local strains of *M. anisopliae* were used against *Coptotermes heimi* Wasmann (Ahmed *et al.*, 2009). The present report describes the efficacy of exotic and indigenous strains of *M. anisopliae*, *Paecilomyces lilacinus*, *Lecanicillium lecani*i and *B. bassiana* against the mustard aphid.

Materials and Methods

Strains of entomopathogenic fungi used during the study: Samples of insects, alive or dead, were collected from agricultural fields of Sindh using brush,

forceps or insect aspirator. The samples were aseptically placed in separate sterile glass vials and test tubes, and taken to the laboratory for isolation and assessment of pathogens. The fungal hyphae were directly isolated from the insect cadavers and inoculated to Potato Dextrose Agar (PDA) medium (Goettel & Inglis, 1997). Other diseased insects with no fungal growth on their body were surface sterilized with 0.1% sodium hypochlorite, rinsed with sterile water, placed in PDA poured Petri Plates and the pure cultures of the fungi growing out from the insects were maintained on Sabroud dextrose agar (SDA) slants. All the locally isolated strains were identified by using key for identification as described by Humber (1997), Humber & Steinkraus (2005); Barnet & Hunter (1988) and Sampson *et al*. (1988). Strains were also received on request from ARS Collection of Entomopathogenic fungal cultures USDA-ARS RW Center for Agriculture & Health, USA and Wahat Al Sehra Nurseries Desert Group Dubai, UAE (Table 1).

Table 1. Isolated or acquired entomopathogenic fungal strains used during the present studies.

S. #	Strain code	Source	Fungi	Region	Host	Habitat
1.	PDRL1187	Desert Group	*B. bassiana*	Dubai, UAE	NA	NA
2.	PDRL922	Local isolate	*L. lecanii*	Khairpur, Pakistan	*Lipaphis erysimi*	Mustard
3.	PDRL1029	Local isolate	*L. lecanii*	Khairpur, Pakistan	*Phenacoccus sp*	Cotton
4.	PDRL526	ARSEF(strain 1912)	*M. anisopliae*	Mexico	*Unknown* sp. *Homoptera*	NA
5.	PDRL738	Local isolate	*M. anisopliae*	Larkana, Pakistan	*Scirpophaga incertulas*	Paddy
6.	PDRL711	ARSEF(strain 3605)	*M. anisopliae*	Pakistan	*Acrotylus* sp.	NA
7.	PDRL812	ARSEF(strain 3846)	*P. lilacinus*	Rampur, India	*Trialeurodes vaporariorum*	NA

Insect rearing & bioassay chamber preparation: Mustard aphids were collected from different canola crop fields and reared on canola plants inside the screen house insect rearing facility for use in further experiments. Petri Plates of 9cm diameter were used as bioassay chambers. Aphids were gently surface sterilized with 0.001% Sodium hypochlorite in water (v/v) for 1 minute and rinsed with sterilized tap water in fine mesh sieve. Fresh leaves of Canola were taken and surface sterilized with 0.005% Sodium hypochlorite for 2 minutes, dipped in 200 ppm solution of penicillin in sterilized water for 1 minute, and then rinsed with sterilized water. A leaf portion (approx: 2"X1" inches), altered by its margins and keeping the mid rib intact, was placed at the center of each Petri plate. Wet and sterilized cotton was placed around the midrib initial end of the leaf for humidity and protection of leaf from dehydration in Petri plate chamber. Initially a brush was used to infest the leaves with aphids but it was observed that insect transfer though brush may also move some died insects resulting in variable pathogenicity results. Therefore, each leaf was kept with a large number of surface sterilized aphids in a 18 cm Petri plate for 30 minutes to allow the active and alive adult insects to move on to the test leaves. These leaves were then transferred to bioassay chambers and numbers of aphids per leaf were counted under a dissecting microscope.

Preparation of spore suspension of fungi: Ten ml of sterilized water with 0.001% Tween 80 was added to each 10 days old culture plate and spore suspension was prepared by gently rubbing the culture surface with the help of a sterilized spatula. Suspension from all plates of a fungus were collected in a 100 ml beaker and mixed thoroughly to get a homogenized mixture. Serial dilution method (Waksman & Fred, 1922) was used to make the dilutions of the spore suspension wherever required.

Laboratory bioassays: In initial experiments, spore suspensions containing $>10^{10}$ c.f.u. ml^{-1} were used to select the potential entomopathogenic isolates. With the help of a 1cc insulin syringe, a drop of spore suspension was placed on each insect in bioassay chambers. Water with 0.001% Tween80 but without fungal spores was used to inoculate the insects in control treatments. After 72 hrs incubation, the insect cadavers were surface sterilized and inoculated in PDA poured Petri plates for re-isolation of the inoculated fungi. On the basis of results of this experiment, strains showing entomopathogenic potential were selected for use in future experiments whereas non-entomopathogenic strains were discarded.

In another experiment, the Spore suspensions of all the selected isolates were diluted to get final, concentrations of 10^9, 10^7, 10^5 and 10^3 spores ml^{-1} and used to evaluate their efficacy by the method described above. The mortality of insects was recorded at 24 hr interval for 5 days in comparison to control insects. The data were used to calculate the LC_{50} and LT_{50} values for each fungal strain using probit analysis for % mortality (Finnay & Stevens, 1948). The experiment was performed at 22±1°C with 10:14 hrs L:D photoperiod. The relative humidity of bioassay chambers was kept at 99±1%.

Screen house bioassays: The experiment was conducted in the month of December and January under natural field condition in a screen house. During these months average relative humidity was 65±1% and average temperature was 19°C±1. Canola cultivar Oscar was used as a test plant since it has no-resistance to test insect pest (Aslam *et al*., 2005). The plants were grown in 24" diam pots under screen house conditions by providing all the standard nutrient and cultural practices except pesticides and fungicides. Each thirty days old canola plant was infested with 15 adult aphids. In the light of laboratory bioassays, 10^7 spores ml^{-1} was selected as the suitable spore concentration for all the test isolates. After 3 days of infestation, 200 ml of spore suspensions were sprayed on each canola plant using a low pressure (<295 psi) sprayer with a nozzle aperture of <300µm. The spray was performed during late evening time. Plants in control

were sprayed with sterilized water containing Tween 80 @ 0.002% (v/v). There were three replicates of each treatment and the experiment was repeated 6 times. The numbers of adult insects on apical raceme, middle and basal leaves in all the treatments were counted before the spray and after each 24hr of the spray for 5 days. Whenever a dead insect was observed in any treatment, it was removed aseptically, observed for fungal growth under low power using a compound microscopes and inoculation on PDA medium for isolation of the pathogen.

Statistical analysis: The data were analyzed by using SPSS19 software for ANOVA and Duncan's Multiple Range Test (DMRT) to analyze the efficacy of different strains and concentrations at $p<0.05$. The Corrected percentage Mortality was obtained by using Henderson-Tilton's formula for heterogeneous populations (Henderson & Tilton, 1955). Time-dose mortality determination for LC_{50} and LT_{50} was also analyzed by Finney's test (Finney & Steven, 1948) using Regression/Probit analysis.

Results

***L. lecanii* isolate PDRL922:** During laboratory bioassay, isolate PDRL922 showed significant mortality of insect population with LC_{50} $5.0x10^3$ spores ml^{-1} (Table 2). A positive correlation between the spore concentration and the mortality of insects was observed since this isolate showed 42, 64, 98 and 100% reduction in populations of insect when used @ 10^3,10^5, 10^7 and 10^9 spores ml^{-1}, respectively ($p<0.001$; Table 3). This isolate was found to be the most virulent strain in laboratory bioassay (Tables 2 & 3; Fig. 2-c,d,f). During the screen house trials, the fungus showed a non-significant reduction in its virulence from 98 (in lab bioassay) to 93% (LT_{50} 1.6 to LT_{50} 1.83 days) when used @ 10^7 spore ml^{-1} (Table 4, Fig. 1).

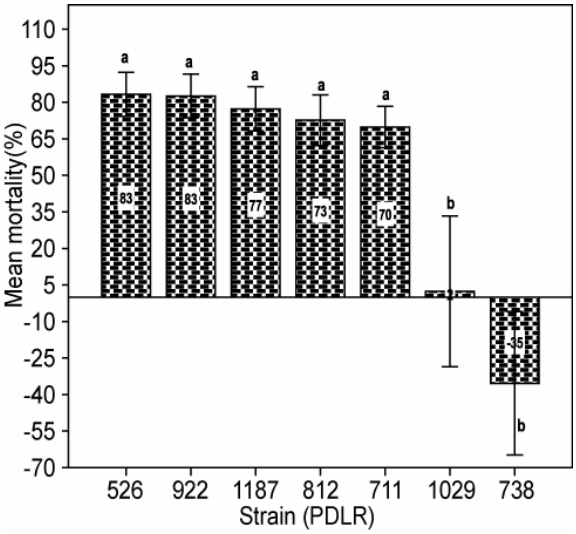

Fig. 1. Mean corrected mortality (%) of insects caused by different test fungal strains after 3 days during screen house bioassay. The bars marked with the same letter are not significantly different according to DMRT at $p<0.05$.

Table 2. LC_{50} for entomopathogenic fungal strains during laboratory bioassay.

S. #	Isolate	LC_{50} (spores ml^{-1})	95% Confidence limit		Slope ± S.E.	Chi-sq	p
			LFL	UFL			
1.	*B. bassiana* PDRL1187	2.7×10^5	3.4×10^4	2.2×10^6	0.49 ± 0.03	53.1	0.001
2.	*L. lecanii* PDRL922	5.0×10^3	1.0×10^2	3.4×10^3	1.1 ± 0.10	63.0	0.001
3.	*L. lecanii* PDRL1029	3.5×10^{20}	-	-	0.2 ± 0.03	36.0	0.011
4.	*M. anisopliae* PDRL526	3.0×10^5	1.4×10^5	4.2×10^8	3.32 ± 0.02	61.8	0.001
5.	*M. anisopliae* PDRL738	1.18×10^{23}	-	-	0.5 ± 0.05	21.8	0.018
6.	*M. anisopliae* PDRL711	2.8×10^8	1.0×10^8	3.4×10^{12}	0.2 ± 0.02	6.7	0.10
7.	*P. lilacinus* PDRL812	5.1×10^3	1.9×10^2	7.0×10^4	0.5 ± 0.04	73	0.001

Table 3. Mortality of insects at various concentrations of fungal strains during laboratory bioassay after three days.

S.#	Strain	% Mortality of mustard aphid in spore suspenssion with different spore concentrations						
		Concentrations						
		10^3	10^5	10^7	10^9	df	F	p
1.	*B. bassiana* PDRL1187	6.8 ± 3.16Bc	31.6 ± 3.5ABb	88.2 ± 4.5Aa	100 ± 0Aa	3	12.4	0.0001
2.	*L. lecanii* PDRL922	42.0 ± 1.7Ab	64 ± 0Aab	98 ± 00Aa	100 ± 0Aa	3	88.2	0.0001
3.	*L. lecanii* PDRL1029	-7.2 ± 11.7Ca	-31.2 ± 25.2Ca	-48.2 ± 37.6Ca	-14 ± 36.9Ca	3	0.38	0.769
4.	*M. anisopliae* PDRL526	12.8 ± 1.6Bc	41.8 ± 4.2ABb	72.6 ± 3.2ABab	96.8 ± 3.2Aa	3	5.8	0.007
5.	*M. anisopliae* PDRL738	-17.9 ± 32.9Cb	-20.4 ± 23.2Cb	-19.4 ± 30.9Cb	25.8 ± 10.5ABa	3	0.755	0.535
6.	*M.anisopliae* PDRL711	0.00 ± 0.0Cc	12.6 ± 4.8Bb	44.2 ± 8.9Ba	48.4 ± 8.5Ba	3	3.3	0.047
7.	*P. lilacinus* PDRL812	36.800 ± 1.9Ab	72 ± 0Aab	100 ± 0Aa	100 ± 00Aa	3	10.8	0.0001
	df	6	6	6	6	-	-	-
	F	9.6	19.2	10.4	9.5	-	-	-
	p	0.000	0.000	0.000	0.000	-	-	-

Mortality values followed by same alphabet (capital letters, in columns for strains and small letter in rows for concentrations) are not significantly different according to DMRT at $p<0.05$. The mean mortality values preceded by (-) negative sign show that the %mortality of test insect is lower than that in control.

Table 4. LT_{50} for fungal strains against mustard aphid in screen house trial when used @10^7 spore ml^{-1}.

S.#	Isolate	LT_{50} (days)	95% Confidence limit		Slope ± S.E.	Chi-sq	p
			LFL	UFL			
1.	*B. bassiana* PDRL1187	2.43	1.80	3.10	1.56 ± 0.31	298	0.001
2.	*L. lecanii* PDRL922	1.83	1.6	2.10	3.6 ± 0.7	15.9	0.313
3.	*L. lecanii* PDRL1029	8.0	6.4	12.9	1.6 ± 0.3	468	0.001
4.	*M. anisopliae* PDRL526	1.82	1.5	2.01	5.2 ± 0.12	61.8	0.262
5.	*M. anisopliae* PDRL738	11.0	10.1	18.3	0.5 ± 0.3	326	0.026
6.	*M. anisopliae* PDRL711	6.8	3.4	8.3	2.2 ± 0.7	526	0.001
7.	*P. lilacinus* PDRL812	2.9	2.3	3.58	2.1 ± 0.3	499	0.001

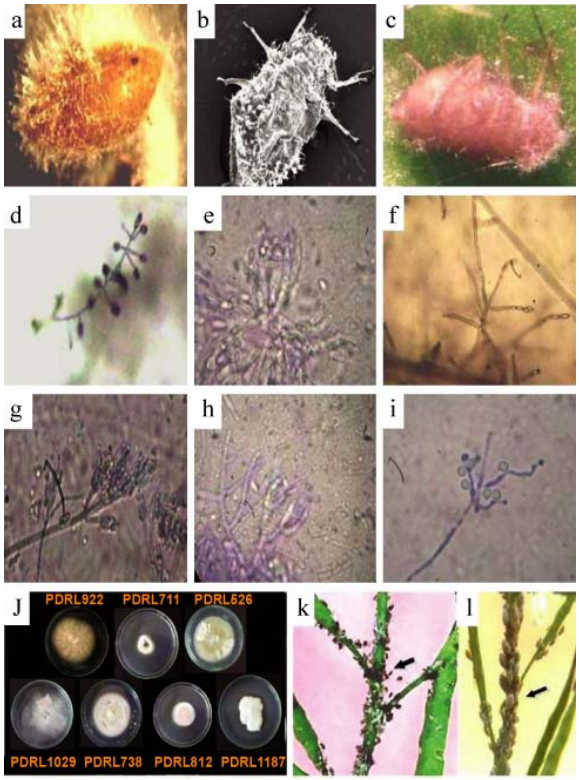

Fig. 2. **(a-c)** The growth of test fungi on mustard aphid after bioassay: **(a)** *P. lilacinus* strain PDRL812, **(b)** *M. anisopliae* strain PDRL526, **(c)** *L. lecanii* strain PDRL922. **(d-i)** Phialides and conidia of test fungi: **(d)** *L. lecanii* strain PDRL922, **(e)** *M. anisopliae* strain PDRL711, **(f)** *L. lecanii* strain PDRL1029, **(g)** *P. lilacinus* strain PDRL812, **(h)** *M. anisopliae* strain PDRL526, **(i)** *B. bassiana* strain PDRL1187. **(j)** The colony growth of the 7 test strains on PDA. **(k)** Died cadavers of aphids on raceme of canola plant after the spray of *P. lilacinus*. **(l)** The infestation of mustard aphid on canola plant before the spray.

***P. lilacinus* isolate PDRL812:** During Laboratory bioassay isolate PDRL812 showed effective mortality of insect population with LC_{50} value of $5.1x10^3$ spores ml^{-1} (Table 2). Isolate PDRL812 significantly reduced ($p<0.001$) the insect population by 36, 72 and 100% when used @ 10^3,10^5 and 10^7 spores ml^{-1}, respectively (Table 3). This isolate was found to be the second most effective pathogen during the laboratory bioassay (Tables 2, 3). In screen house trial, it showed a reduction in virulence from 100 (in lab bioassay) to 73% (LT_{50} 1.62 to LT_{50} 2.9 days) at 10^7 spore ml^{-1} concentrations (Table 4, Fig. 1 & 2-k,l).

***B. bassiana* isolate PDRL1187:** During Laboratory bioassay isolate PDRL1187 showed effective mortality of insect population with LC_{50} value of $2.7x10^5$ spores ml^{-1} (Table 2). Isolate PDRL1187 reduced ($p<0.001$) the insect population by 6.8, 31.6, 88.2 and 100% when used @ 10^3, 10^5, 10^7 and 10^9 spore ml^{-1}, respectively (Table 3). This isolate was found to be the 3rd most virulent strain in laboratory bioassay (Tables 2, 3). Virulence of Strain PDRL1187 in screen house trial decreased 77% (LT_{50} 1.8 to LT_{50} 2.43 days) at 10^7 spore ml^{-1} concentrations as compared to 88% during lab bioassay (Table 4, Fig. 1).

***M. anisopliae* isolate PDRL526:** During Laboratory bioassay isolate PDRL526 showed effective mortality of insect population with LC_{50} value of $3.0x10^5$ spores ml^{-1} (Table 2). Isolate PDRL526 consistently reduced ($p<0.001$) the insect population by 12.8, 41.8, 71.6 and 96.8% when used @ 10^3, 10^5, 10^7 and 10^9 spore ml^{-1}, respectively (Table 3). This isolate was found to be the 4th most virulent strain in laboratory bioassay (Tables 2, 3 & Fig.2-h). Virulence of strain PDRL526 in the screen house trial increased to 83% (LT_{50} 2.1 to LT_{50} 1.82 days) at 10^7 spore ml^{-1} concentrations as compared to 72.6% during lab bioassay (Table 4, Fig. 1).

***M. anisopliae* isolate PDRL711:** During Laboratory bioassay *M. anisopliae* isolate PDRL711 appeared to be the least effective in causing the insect mortality and showed the LC_{50} value of $2.8x10^8$ spores ml^{-1} (Table 2). Isolate PDRL711 reduced ($p<0.05$) the insect population by 0.0, 12.6, 44.2 and 48.4% when used @ 10^3, 10^5, 10^7 and 10^9 spore ml^{-1}, respectively (Table 3). Virulence of Strain PDRL711 during screen house trial increased to 70% (LT_{50} 3.2 to LT_{50} 6.8 days) as compared to 44.2% during lab bioassay at 10^7 spore ml^{-1} concentrations (Table 4, Fig. 1). It was observed that the LT_{50} value of the strain increased during screen house bioassay.

***M. anisopliae* isolate PDRL738 and *L. lecanii* isolate PDRL1029:** Both were found to be avirulent during the laboratory (LT_{50} >12 days for both the isolates) as well as screen house bioassays (LT_{50} 8 and 11days, respectively) when used @ 10^7 spore ml^{-1}.

Discussion

The laboratory and screen bioassays showed that entomopathogenic fungal strains PDRL922, PDRL812, PDRL1187 and PDRL526 were highly effective in the control of mustard aphid. The strain PDRL711 was found least effective since it has a long LT_{50} period. *M. anisopliae* and *B. bassiana* are known entomopathogenic fungi and have shown effective virulence against the mustard aphid. Araujo *et al*., (2009) have reported that *B. bassiana* caused 90% mortality after 4.4 days at 10^7 spore ml^{-1} concentrations whereas isolate PDRL1187of *B. bassiana* used during the present studies produced 88% mortality within 3 days. Araujo *et al*., (2009) also reported that some isolates of *M. anisopliae* showed 64% virulence against mustard aphid population after 3.8 days whereas in the present study, isolate PDRL526 of *M. anisopliae* showed 72% mortality within 3 days. It shows that the isolates used during the present studies are more virulent as compared to the isolates used by Araujo *et al*., (2009). Parmar *et al*., (2008) reported that *L. lecanii* showed 77.16% mortality after 10 days against *L. erysimi* when used @ 10^5 spore ml^{-1}. During the present studies, *L. lecanii* strain PDRL922 was found more effective since it produced 100% mortality of mustard aphid after 3 three days. Butt *et al*., (1994) reported that various strains of *M. anisopliae* and *B. bassiana* caused 100% mortality in *L. erysimi* and *Myzus persicae* after 4 days that corroborates well with the results of the present studies. Saranya *et al*., (2010) reported *L. lecanii*, *M. anisopliae* and *B. bassiana* strains caused 100, 83.3 and 61.5% mortality, respectively of cow pea aphid (*Aphis craccivora* Koch) after 7 days when used @10^7 spores ml^{-1}. During the present studies, *L. lecanii* isolate PDRL922, *M. anisopliae* isolate PDRL526 and *B. bassiana* isolate PDRL1187 showed 98, 72 and 88% mortality of mustard aphid after 3 days @ 10^7 spore ml^{-1}. The difference in virulence of the isolates may be attributed to genetic variation in fungal strains, difference in bioassay methods, aphid species and/or to abiotic and biotic effects such as sunlight (Inglis *et al*., 1993; Braga *et al*., 2002), temperature (Noma & Strickler, 1999), humidity (James *et al*., 1998), Phylloplane (Shipp *et al*., 2003) genetic variability of fungal stain, formulation and application methods (Shah & Butt, 2005).

The results of the present studies show that *P. lilacinus* strain PDRL812, *L. lecanii* strain PDRL922, *B. bassiana* strain PDRL1187, *M. anisopliae* strains PDRL526 and PDRL711 when used @ 10^7 c.f.u. ml^{-1} were highly virulent against aphid population on canola plant. It is interesting to note that *M. anisopliae* strains PDRL526 and PDRL711 used during this study have also been reported to produce 100% mortality of Hibiscus Mealy bug (*Maconellicoccus hirsutus)* adults and instars after 7 and 4 days (Ujjan & Shahzad, 2007). *P. lilacinus* strain PDRL812 (ARSEF3846) that showed good control of mustard aphid during the present studies was found slightly virulent against white fly *Trialeurodes vaporariorum* by Gokce (2005). Similarly, strains of *L. lecanii*, *B. bassiana* and *Paecilomyces* sp., have also been reported to cause 75 to 100% mortality in cotton aphid (*Aphis gossypii)* (Kim *et al.*, 2001). It appears that the use of such strains can provide protection against more than one insect pest. Development of cheap and effective methods for mass production of the biocontrol agents to be used as myco-pesticides will play a significant role in sustainable development of organic based IPM agriculture practices resulting in reduction in health and environmental hazard caused by chemical pesticides.

Acknowledgements

The exotic strains used during the present studies were kindly provided by ARS Collection of Entomopathogenic fungal cultures, USDA-ARS RW Center for Agriculture & Health, USA, and Wahat Al Sehra Nurseries Desert Group Dubai, UAE that is gratefully acknowledged.

References

Ahmed, S., M.R. Ashraf and A. Hussain. 2009. Pathogenicity of isolates of *Metarhizium anisopliae* from Gujranwala (Pakistan) against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae). *Int. J. Agri. Bio.,* 11-6: 707-7.

Ambethger. 2009. Potential of Entomopathogenic fungi in insecticide resistance management (IRM): A review. *J. Biopesticides*, 2(2): 177-193.

Anonymous. 2010. Edible Oil Estimates, *Economic Survey of Pakistan, Finance Division Government of Pakistan*, 2010-11: 21.

Araujo, Jr. J.M.D., J. M. Edmilson and J. V. Oliveira. 2009. Potencial de solados de *Metarhizium anisopliae* e *Beauveria bassiana*e do Óleo de Nim no Controle do Pulgão *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae) *Neotropical Entomology*, 38(4): 520-525.

Asi, M.R., M.H. Bahir, M. Afzal and Saqib. 2009a. Effect of conidial concentration of entomopathogenic fungi on mortality of cabbage aphid, *Brevicoryne brassicae* L. *Pak. J. Life Soc. Sci.*, 2: 175-180.

Asi, M.R., M.H. Bashir, J. H. Mirza, M. Afzal and Imran. 2009b. *In vitro* efficacy of entomopathogenic fungi against cabbage aphid, *Brevicoryne Brassicae* L. *Pak. Ento.*, 31(1): 43-47.

Asi, M.R., M.H. Bashir, M. Afzal, M. Ashfaq and S.T. Shahi. 2010. Compatibility of entomopathogenic fungi *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* with selective insecticides. *Pak. J. Bot.,* 42(6): 4207-4214.

Aslam, M., M. Razaq and A. Shahzad. 2005. Comparison of different canola (*Brassica napus* L.) varieties for resistance against cabbage aphid (*Brevicoryne brassicae* L.). *Int. J. Agri. & Bio.*, 7(5): 781-782.

Barnet, H.L. and B.B. Hunter. 1988. *Illustrated Genera of Imperfect Fungi.* (4th Ed.).American Phytopathological Society.

Braga, G.U.L., E.N.R. Drauzio, D. F. Stephan, C.D. Miller, A.J. Anderson and D.W. Roberts. 2002. Damage and recovery from UV-B exposure in conidia of the entomopathogens *Lecanicillium lecanii* and *Aphanocladium album*. *Mycologia*, 94(6): 912-920.

Butt, T.M., L. Ibrahim, B.V. Ball and S.J. Clark 1994. Pathogenicity of the entomogenous fungi *Metarhizium anisopliae* and *Beauveria bassiana* against crucifer pests and the honey bee. *Biocontrol Sci. & Tech*., 4(2): 207-214.

Emden, V.H.F. and R. Harrington. 2007. *Aphids as crop pests.* CABI Publishing, London. Nosworthy Way, Wallingford, Oxfordshire, UK. 717pp.

Fargues, J. 1975. Consideration of the nature of experimental utilization combine's *Beauveria bassiana* and insecticides a dose reduced against *Leptinotarsa decemlineata*. *Ann. Zool. Ecol. Anim.*, 7: 247-264.

Faria, M.R and S.P. Wraight. 2007. Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Bio. Cont.,* 43: 237-256.

Farooq, A. 2007. *Insect Pest of Canola (Barasica napus) and their management*. Ph.D. Thesis. Bahauddin Zakrya University, Multan, Pakistan.

Finney, D.J. and W.L. Stevens. 1948. A table for the calculation of working probits and weights in probit analysis. *Biometrika,* 35(1-2): 191-201.

Franz, J.M. 1961. Biological control of pest insects in Europe. *Annual Review of Entomology*, 6: 183-200.

Goettel, M.S and G.D. Inglis. 1997. Fungi: Hyphomycetes. In: *Manual of Techniques in Insect Pathology.* pp. 213-247. (Eds.): L.A. Lacey. Academic Press, London.

Gokce, A. 2005. Pathogenicity of *Paecilomyces* spp., to the glass house whitefly, *Trialeurodes vaporariorum*, with some observations to the fungal infection process. *Turk J. Argic.,* 29: 331-339.

Hall, I.M. 1963. Microbial Control. In: *Insect Pathology, An Advanced Treatise* 477-511. (Eds.): E.A. Steinhaus. Vol. 2. Academic Press, New York.

Henderson, C.F. and E.W. Tilton. 1955. Test with acaricides against the brown wheat mite. *J. Econ. Entomol.,* 48(2): 157-161.

Humber, R.A. 1989. Synopsis of a revised classification for the Entomophthorales (Zygomycotina). *Mycotaxon,* 34: 441-460.

Humber, R.A. 1997. Fungi Identification. In: *Manual of Techniques in Insect Pathology.* pp. 153-185. (Eds.): L.A. Lacey, Academic Press London.

Humber, R.A. and D.S. Steinkraus. 1998 updated 2005. Entomopathogenic Identification. In: *Proceedings of workshop*.(Eds.): American Phytopathological Society and Entomological society of America. http://arsef.fpsnl.cornell.edu .

Inglis, G.D., M.S. Goettel and D.L. Johnson. 1993. Persistence of the entomopathogenic fungus, *Beauveria bassiana*, on phylloplanes of crested wheat grass and alfalfa. *Biol. Control,* 3: 258-270.

Irshad, M. 2001. Aphids and their biological control in Pakistan. *Pak. J. Bio. Sci.,* 4(5): 537-541.

James, R.R., B.A. Croft, B.T. Shaffer and B. Lighthart. 1998. Impact of temperature and humidity on host-pathogen interactions between *Beauveria bassiana* and a coccinellid. *Environ. Entomol.,* 27: 1506-1513.

Khetan, S.K. 2001. *Microbial pest control*. Marcel Dekker, Inc. NY, USA.

Kim, J.J., M.H. Lee, C.S. Yoon, H. Kim, J. Yoo and K.C. Kim. 2001. Control of cotton aphid and greenhouse whitefly with a fungal pathogen. *Bulletin, Korean National University, Kwangju,* 7-14.

Laird, M. 1962. *Coelomomyces fungi, an important group of mosquito parasites*. World Health Organization, Geneva, Switzerland.

Lipson, M. 1997. Searching for the O Word: What's Wrong with this Picture? *Organic Farming Research Foundation Information Bulletin,* 4:(1): 12-14.

Love, H.K., G. Rakow, J.P. Raney and R.K. Downey. 1990. Genetic control of 2-propenyl and 3-butenyl glucosinolate synthesis in mustard. *Can. J. Plant Sci.,* 70: 425-429.

Meitkiewski, R.T., J.K. Pell and S.J. Clark. 1997. Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: Field and laboratory comparisons. *Biocontrol Sci. Technol.,* 565-757.

Noma, T and K. Strickler. 1999. Factors affecting *Beauveria bassiana* for control of Lygus bug (Hemiptera: Miridae) in alfalfa seed fields. *J. Agric. Urban Entomol.,* 16(4): 215-233.

Parmar, G. M., M.N. Karpadia and B.K. Davda, 2008. Bio-efficacy and cumulative effect of *Verticillium lecanii* (Zimmerman) against *Lipaphis erysimi* (Kaltenbach) on mustard *Internat. J. Agric. Sci.,* 4: 204-206.

Pedro, M.O.J., E.H. Neves, T.T. Paulo and M.J.R. Alcides. 2001. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotropical Entomology,* 30(2): 263-268.

Rehner, S.A. 2005. *Phylogenetics of the insect pathogenic genus Beauveria.* pp. 3-27. In: *Insect-Fungal Associations: Ecology and Evolution*, (Eds.): F.E. Vega and M. Blackwell, Oxford University Press London.

Roberts, D.W. and R.J. St. Leger. 2004. *Metarhizium* spp., cosmopolitan insect-pathogenic fungi: Mycological aspects. *Adv. Appl. Microbiol.,* 54: 1-70.

Sampson, R.A., H.C. Evans and J.P. Latge'. 1988. *Atlas of Entompathogenic Fungi.* pp.1-187. (Eds.): Springer_Verlag, Berlin, Heidelberg. New York.

Saranya, S., R. Ushakumari, S. Jacob and B.M. Philip. 2010. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *Jr. of Biopesticides*, 3(1): 138-142.

Shah, F.A and T.M. Butt. 2005. Influence of nutrition on the production and physiology of sectors produced by the insect pathogenic fungus *Metarhizium anisopliae. FEMS M.bio. Letters,* 250: 201-207.

Shipp, J. L., Y. Zhang, D.W.A. Hunt and G. Ferguson. 2003. Influence of humidity and greenhouse microclimate on the efficacy of *Beauveria bassiana* (Balsamo) for control of greenhouse arthropod pests. *Env. Entomol.,* 32: 1154-1163.

Steinhaus, D.C. 1958. Stress as a factor in insect disease. *Proceedings of 10th International Congress of Entomology,* 4: 725-730.

Ujjan, A.A and S. Shahzad. 2007. Pathogenicity of *Metarhizium anisopliae* var *acridum* strains on pink hibiscus mealy bug (*Maconellicoccus hirsutus*) affecting cotton crop. *Pak. J. Bot.,* 39(3): 967-973.

Waksman, S.A. and E.B. Fred. 1922. A tentative outline of the plate method for determining the number of microorganisms in the soil. *Soil Sci.,* 14: 27-28.

(Received for publication 20 August 2011)