Pakistan J. Zool., vol. 47(2), pp. 351-360, 2015.

Efficiency of *Metarhizium* spp. (Sorokīn) Strains and Insecticides Against Cotton Mealybug *Phenacoccus solenopsis* (Tinsley)

Aziz Ahmed Ujjan,[1]* Muhammad Ali Khanzada,[2] A.Q. Mahar[1] and Saleem Shahzad[3]
[1]*Department of Botany, Shah Abdul Latif University, Khairpur-66020, Pakistan*
[2]*Department of Plant Protection, Sindh Agriculture University, Tando Jam-70060, Pakistan*
[3]*Pest and Disease Research Laboratory (PDRL), Department of Agriculture & Agribusiness Management, University of Karachi, Karachi-75270, Pakistan*

Abstract.- We conducted a study to assess the insecticidal potential of the Hypocreales entomopathogenic fungi (epf) *Metarhizium* spp. strains, insecticides and their mixture for control of cotton mealybug (CMB) under laboratory, screen house and field conditions. The strain PDRL526 was the most effective amongst the strains of *Metarhizium* spp. at laboratory bioassays. It caused 50% mortality (LT_{50} value) of adult cotton mealybug at 5.2 days (LT_{50} value = median lethal time) after the application of $1.57x10^5$ spore/cm^2 ($6.3x10^{12}$ spores/acre) inside the bioassay chamber. Therefore, the strain PDRL526 was selected to study at the screen house and field trials. The insecticide Lambda-Cyhalothrin was highly effective with 50% lethal concentration (LC_{50} value) (1.12 µg/ml) followed by Acetamiprid (1.17 µg/ml), Abamectin (1.62 µg/ml), Imidacloprid (1.67 µg/ml), Chlorpyrifos (2.09 µg/ml) and Bifenthrin (3.05). The insecticide Imidacloprid showed the best compatibility (95.2%) to the strain PDRL526; therefore, Imidacloprid was selected for screen house and field trials. The strain caused adult CMB mortality after (LT_{50}) 13.8 and 19.6 days by using 6.3 $x10^{12}$ spores/acre, under screen house and field conditions, respectively. The strain's application in combination with insecticide Imidacloprid (20 g a.i. /acre + $6.3x10^{12}$ spores/acre) showed a positive toxicity/virulence to CMB population at screen house and field trials with LT_{50} 6.57 and 8.4 days, respectively. Along with the pest mortality, the yield of seed cotton/plant, increased with the spray of spores of the strain PDRL526, alone or in combination with Imidacloprid, at screen house and field trials as compare to control treatments. The study confirmed that *M. anisopliae* strain PDRL526 is effective against CMB.

Key words : Mycoinsecticides, entomopathogenic fungi, neonicotinoid insecticides, biological control.

INTRODUCTION

CMB, the cotton mealybug (*Phenacoccus solenopsis* Tinsley), had not posed any severe threat as crop pest until the end of 1990, when Watson and Chandler (2000) identified and reported its pest habit on several plants. Its pest habit was reported for the first time in Pakistan (South Asia) in the year 2005 and became a common (acclimatized) pest in Pakistan (Anonymous, 2008a) and India (Anonymous, 2008b; Nagrare *et al*., 2008). It caused heavy losses in cotton belts (Sindh and Punjab locations) in Pakistan (Anonymous, 2006, 2008c; Zaka *et al*., 2006; Kakakhel, 2007). CMB is difficult to control with low doses of chemical insecticides, because it has complex layer of wax that protects it against the contacts with pesticides. Therefore combinations between insecticides and biocontrol agents which can biodegradate this complex layer of wax (*e.g.* using specific enzymes) could present efficient results for CMB control (Fuchs *et al*., 1991).

The habit of CMB is sap sucking, which is also a defensive mode of nutrition against chemical contact pesticides (Fuchs *et al*., 1991), therefore the over doses are required to check insect pests. Since the hazards of insecticides and their residue in agro-products posed the concerns regarding human environment and health, the researchers look for organic farm or pesticide residue free products. Several remedies have been probed under biocontrol of diseases and pests of crops (Pimentel, 2009). Amongst the biocontrol agents, entomopathogenic fungi (epf) serve as mycoinsecticide (Faria and Wraight, 2007; Brand *et al*., 2012).

Members of the genus *Metarhizium* (Metschnikoff) Sorokin, also called green muscardine fungi, have been used against wheat chafer beetles *Anisoplia austriaca* and sugar beet curculio, *Cleonus punctiventris* (Metschnikoff,

* Corresponding author: ujjjann@gmail.com
0030-9923/2015/0002-0351 $ 8.00/0

1884; Lord, 2005). At present, the world has about 60 commercial mycoinsecticides based on *Metarhizium* spp. (Faria and Wraight, 2007); the most strains of *M. anisopliae* have a broad and divergent host range. *M. anisopliae* strains were reported effective against potato aphid (*Myzus persicae*), cabbage seed weevil (*Centrorhynchus assimilis*), cabbage flea beetle (*Psylliodes chrysocephala*), mustard beetle (*Meligethes aeneus*) (Butt *et al*., 1998) and mustard aphid (*Lipaphis erysimi*) (Ujjan and Shahzad, 2012). *M. anisopliae* was also used against black wine weevil (*Otiorhynchus sulcatus*) a pest of various ornamentals (Vestergaard *et al*., 1995). Some studies reported the potential of epf against *P. solenopsis* (Makadia *et al*., 2009; Kumar *et al*., 2012; Banu *et al*., 2010). Although, there is appreciable amount of work carried out on other biocontrol agents, use of epf against CMB is unexplained in Pakistan. Therefore, the work was carried out to assess the insecticidal potential of epf strains for their assessment against CMB under laboratory, screen house and field conditions alone and with combination of the suitable insecticide formulation.

MATERIALS AND METHODS

Collection of samples

The team collected samples from Sindh, Pakistan. Live and dead insects with the symptoms of diseases were collected and processed for fungal isolation following the procedure of Goettel and Inglis (1997). The fungi were identified after Samson *et al*. (1988), Humber (2012), Barnett and Hunter (1988) and Domsch *et al*. (1980). The known isolates of epf were received from ARS Collection of Entomopathogenic fungal cultures, USDA-ARS RW Center for Agriculture and Health, USA. Every strain that was isolated from insect cadavers was allotted a collection number with respect to its host, collection time/date and area and stored at 20ºC after pure colony inoculation inside PDA poured slants.

Insect rearing

Insects were cultured on their host plants (cotton). Plant saplings grown in 15.24x20.32 cm earthen pots filled with loamy soil and humified cow dung (4:1 ratio). Each pot was kept at 14:10 h (light: dark) photoperiod. The seedlings were placed in a large chamber and covered with a fine net cloth supported by steel rod scaffoldings (76.2x17.7 cm) under natural light conditions of screen house. The pots were managed with cultural agronomy. The healthy adult insects were transferred to the saplings. The heavily infested plants were transferred to the laboratory.

Laboratory bioassay

The insect infestation on plant was washed with 0.01% sodium hypochlorite solution followed by sterilized distilled water. The required numbers of insects of respective life stages were transferred to bioassay chambers by using (zero size) camel hairbrush and nylon tip forceps. The entomopathogenic fungal spores were collected from 15 days old culture growing on PDA medium. The culture added with 5 ml sterile water and 0.02% Tween80 (v/v). The spores were slightly eroded with spatula and the spore containing solution transferred to a test tube. Hemocytometer used to count the epf spores per ml and the numbers of spores/ml were adjusted through dilution formula (Goettel and Inglis, 1997). The known number of insects per bioassay chamber (9 cm diameter or 63.6-cm^2 area Petri plate) were infected with 1 ml of epf spores using an insulin syringes BD^{TM} of 26 gauge needle. This micro spray technique adjudicated to consume 1ml spore suspension per bioassay chamber. Each strain was applied to insects inside the bioassay chambers with $1x10^7$ spores' concentrations ($1.57x10^5$ spore/cm^2 or $6.3x10^{12}$ spores acre). Each treatment was replicated 5 times. Another set of treatments was assayed with water and 0.02% Tween80 solution as control. The insect population of the each treatment were noted every day for live and dead numbers. The percent mortality calculated through Abbott formula in comparison with control mortality (Abbott, 1925). Time dose mortality probit analysis was applied for the most probable lethal time of the insect. The epizootic symptoms of cadavers inside bioassay chambers were observed and analyzed by microscopic examination and inoculation of insect on PDA medium. After the confirmation of this

preliminary test, the isolate was marked for further bioassays and experiments. The known concentration of insecticides were assayed on the insect population inside the bioassay chamber and the number dead/alive insects were noted after24 h and the data was analysed for determination.

Compatibility test

For compatibility assessment of insecticides, the amount of water and pesticides' concentrations were used according to the recommended concentrations of the insecticide. The epf strain was examined for compatibility with chemical insecticides under the reference to Neves *et al*. (2001).

Screen house bioassay

The screen house assays were conducted at Pest and Disease Laboratory Green House facility, University of Karachi, Pakistan. The screen house was protected with a steel net of 2.5 cm^2 and nylon net cover of 2 mm^2 for protection from birds, insects and light. The large size (2 $feet^2$) earthen pots were filled with sun dried loamy soil and cow dung manure (3:1 w/w). The pots were irrigated two days before sowing the seed. The variety of cotton (Variety NIAB 78) was selected based on their known susceptibility to test insect. The cottonseeds were soaked in water for 12 h before the sowing. Three young seedlings were kept intact and remaining plants were thinned after a foot height growths, remaining two were also thinned and one plant was maintained per pot. All the recommended fertilizers and irrigations were followed. Each plant was infested with 05 female insects, 15 days earlier to spray regimes. The spray regimes were started in June 2010. The combined effect of epf and chemical pesticides were assessed by combined application of selected epf spore concentration with chemical pesticide, in the light of laboratory bioassay results of the fungal strains and pesticides. The best compatible pesticide was selected for synergistic application in screen house bioassays. Tween80 (0.02%) aqueous solution was mixed with epf spores grown on broken rice grains through single-phase fermentation (SPF). The numbers of harvested spores per gram of substrate are counted by using hemocytometer and the required concentration made over. Each treatment was sprayed with epf spores ($6.30x10^{12}$ spores/acre); the epf spores concentration made in hand carry sprayers. Each sprayer was used against five replicates of single treatment. The control plants were sprayed with the spores and pesticide free Tween80 (0.02% v/v) solution. The recommended doses of the most compatible insecticide (Imidacloprid) were used for CMB (20 g/acre). Population of insects on plant was counted before the spray and the changes in populations noted at different time intervals (01, 05, 10, 20 and 30 days) after the spray. The adult CMB population was counted on 10 cm length on twig and for instars; CMB leaf midrib area of test plant was counted. At the end of season, plants were harvested and their lint + seeds from dehiscent capsule (cotton) were collected and weighed. The differences between control and among treatments were analyzed. The experiment was completely randomized designed and the mortality percentage was corrected using Henderson and Tilton (1955) formula. The LT_{50} of the treatments were analyzed using probit analysis..

Field bioassay

The field applications were carried from April to September 2011. The test crop was cultivated in three plots with area of (20x10 feet) for each test. The plot-to-plot distance was about 12 feet. The soil and seedbed was prepared and seed sowed as per recommendations and according to agronomic protocol and procedures. After germination, 30 plants were maintained in each block with 2 feet plant-to-plant and 2.5 feet row-to-row distance. All plants were artificially infested with CMB five females per plant prior to 15 days of the spray treatments. Each plot was sprayed with the epf using low volume sprayer of 16L capacity, with prevention to cross contamination. The fungal spores and pesticide concentrations were applied in same amount as in screen house bioassay. Each plot was sprayed with insecticide (a.i. 20g/acre or 91.8 mg/plot) and epf spores ($6.3x10^{10}$ spore/acre or $2.89x10^{12}$/plot) in combined or alone, preparations in aqueous dilution. The spore treatments were also added with 0.02% Tween 80 as emulsifier. A plot was sprayed with the same volume of 0.02% Tween80 sterilized aqueous solution as control. The

Table I.- Details of different *M. anisopliae* isolates/strains with reference to their collection and virulence (LT_{50}) to cotton mealybug (*Phenacoccus solenopsis*) adults at laboratory bioassays.

Strain code	LT_{50} ±SE days	Source	Fungi	Region	Host	Habitat
PDRL18	17.69±2.1	Local isolate	*M. anisopliae*	Karachi, Pakistan	*Phenacoccus* sp.	Cotton
PDRL 116	13.5±1.3	Local isolate	*M. anisopliae*	Karachi, Pakistan	*B. tabaci*	Okra
PDRL 129	22.40±3.2	Local isolate	*M. anisopliae*	Khairpur, Pakistan	*B. tabaci*	Cotton
PDRL 137	15.55±2.9	Local isolate	*M. anisopliae*	Larkana, Pakistan	Rice stem borer adult	Paddy
PDRL 174	16.99±7.4	Local isolate	*Metarhizium* sp.	Hyderabad, Pakistan	*B. tabaci*	Cotton
PDRL 220	18.96±3.8	Local isolate	*Metarhizium* sp.	Khairpur, Pakistan	*B. tabaci*	Cotton
PDRL 269	14.88±2.5	Local isolate	*Metarhizium* sp.	Khairpur, Pakistan	*Phenacoccus* sp.	Cotton
PDRL 526	5.24±4.6	ARSEF (strain 1912)	*M. anisopliae*	Mexico	Homoptera	NA[1]
PDRL 711	10.65±3.2	ARSEF (strain 3605)	*M. anisopliae*	N.A., Pakistan	*Acrotylus* sp.	NA
PDRL 738	11.06±8.3	Local isolate	*M. anisopliae*	Larkana, Pakistan	*Scirpophaga incertulas*	Paddy
PDRL 744	15.84±5.8	Local isolate	*Metarhizium* sp.	Khairpur, Pakistan	*Chilo infuscatellus*	Sugar cane
PDRL 1043	15.61±6.3	ARSEF (strain 1729)	*M. pingshaense*	Tamil nidu, India	*Nilaparvata lugens*	Green house

[1] Not available

number of live adults and instars (CMB) population was randomly counted on 1 cm twigs (for CMB adults) of five plants in the plot before spray (day 1) and after 5, 10, 20 and 30 days of spray regimes. The differences of populations were calculated between control and treatments. Percent mortality was corrected using Henderson and Tilton formula (1955). The seed cotton yield of treated and control plants was also noted.

RESULTS AND DISCUSSION

Epf isolates

There were 12 *Metarhizium* spp. strains collected at laboratory culture collection, of which 09 were locally isolated and 03 received from abroad (Table I). The local isolates consisted of a huge number (1183) of non-target fungi *i.e. Aspergillus* sp., *Cladosporium* sp., *Alternaria* sp., *Fusarium* sp., *Nigrospora* sp., *Dreschslera* sp. and others, from insects. The isolation and assessment work emphasized over well-reported epf. The fungal contaminant issue reported as common hurdle (Goettel and Inglis, 1997).

Lab. bioassays

PDRL526 *M. anisopliae* caused higher virulence (LT_{50} 5.24 days) than other 12 *Metarhizium* spp. strains (Table I). The strain was therefore selected for further quality and virulence tests in screen house and field conditions on CMB populations (*in vivo*) infested on cotton plants. There are some reports available about the strains of *M. anisopliae* that were virulent to papaya mealybug (*Paracoccus marginatus*) and CMB *in vitro* conditions (Anonymous, 2010; Kumar *et al.,* 2012; Banu *et al.,* 2010; Nagrare *et al.,* 2011). The report supports the present study. Other 11 strains of *Metarhizium* spp. were virulent with lower results against the CMB (Table I). This is due to their lower pathogenic attributes, as it is known factor that the different epf strains have different effects on same host insect, even if the strains are of the same species.

Insecticide toxicity and compatibility

Insecticide toxicity test was conducted to CMB using six insecticides of different groups for LC_{50} after 24 h (Table II). The insecticides were assessed for the selection of the best suitable insecticide for combine/synergistic application to epf at screen house and field bioassays. The insecticide lambda-cyhalothrin found highly toxic to CMB with LC_{50} 1.12 ppm followed by acetamiprid (1.17 µg/ml), abamectin (1.62 µg/ml), imidacloprid (1.67 µg/ml), chlorpyrifos (2.09 µg/ml) and bifenthrin (3.05 µg/ml) against CMB adult population inside bioassay chambers after 24 h of application (Table II).

Insecticide imidacloprid found more compatible to epf strains, when it examined for its effect on fungal colony and spore growth on culture media poised with its the recommended dose (Table III). It showed higher compatibility (95%) to the epf

strain (Table III), therefore the insecticide preferred to be studied in screen house and field bioassays regimes.

Table II.- LC_{50} of pesticide concentrations at 24 h time interval, against cotton mealybug (*P. solenopsis*) adults in laboratory assays.

Pesticide	LC_{50} (µg/ml)	95% confidence limit		Chi^2 (df^a=48)
		Lower	Upper	
Abamectin	1.624	1.454	1.829	366.6*
Acetamiprid	1.170	0.761	1.585	4941.0*
Bifenthrin	3.054	2.615	3.787	252.0*
Chlorpyrifos	2.098	2.009	2.197	45.5*
Imidacloprid	1.671	1.519	1.852	286.2*
Lambda Cyhalothrin	1.127	.892	1.367	1771.8*

[a] Statistics based on individual cases differ from statistics based on aggregated cases.
* Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits.

Table III.- Percent compatibility (T-value) of *M. anisopliae* strain PDRL526 with chemical insecticides.

Insecticide (recommendation µg/ml*)	PDRL526
Abamectin (10)	65.57±5.2b
Acetamiprid (25)	86.8±12.1a
Bifenthrin (25)	38.0±2.6d
Chlorpyrifos (400)	74.5±9.3b
Imidacloprid (200)	95.2±6.8a
Lambda Cyhalothrin (10)	55.7±1.2c
Control (00)	100±0.1a

*The recommended insecticide dilution was formulated in accordance to 100 L of water per acre in compatibility test. The values followed by same letters are not significantly different at Duncan's multiple range test, $p<0.05$

Ahsan (2007) reported that insecticide abamectin and lambda cyhalothrin showed LC_{50} 0.68 and 1.2 µg/ml against CMB adults that supports our results. No studies are available on the effect of imidacloprid, acetamiprid, bifenthrin and chlorpyrifos against *P. solenopsis*.

Imidacloprid was found highly compatible at 200 µg/ml with epf strains in the present study. There are several reports on compatibility of Imidacloprid with epf strains (Kim and Kim, 2007; Alizadeh *et al*., 2007; James and Elzen, 2001; Quientela and McCoy, 1998). The insecticides imidacloprid, therefore, holds promise for combined use in integrated pest management (IPM) strategies with epf.

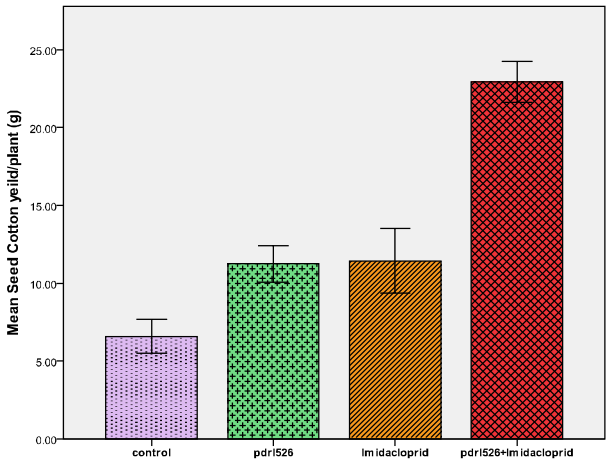

Fig. 1. Seed cotton yield of treated cotton plants with *M. anisopliae* strains PDRL526 alone and in combination with insecticide or insecticide alone under screen house condition.

Screen house bioassays

The strain sustained stress on CMB population with negotiable variation of virulence (LT_{50} 13.8 days) under screen house conditions. The strain protected the plant which benefited and increased seed cotton yield after treatments (11.5 g/plant) as compared to control (7.0 g/plant) (Fig. 1). The synergistic effect of insecticide and epf strains geometrically increased the insect mortality and caused decrease in LT_{50} values (6.5 days) under screen house conditions. The strain boosted cotton yield (11.5 g/plant) as compared to control treatments (7.0 g/plant), and the plants yielded double under synergistic effects of insecticide and epf (23 g/plant) (Fig. 1, Table IV).

The virulence of the strain reduced at screen house and consequent field trials (Table I, IV &V), Kumar *et al.* (2012) reported same observations that the virulence of epf strains decreased during *in vivo* bioassays against another group of insect pests, which supports the present study. The difference in virulence might be attributed to the higher abiotic

and biotic stress like sunlight (UV radiation), wind, humidity deficits, symbiotic organisms at insect body and phyloplane and allelopathic compounds (Thomas and Jenkins, 1997; Hallsworth and Magan, 1994; Rangel *et al*., 2004; Inglis *et al*., 1997; Goettel and Inglis, 1997; St-Leger, 2008).

Table IV.- LT_{50} caused by *M. anisopliae* strain PDRL526 concentration and chemical pesticides $6.30x10^{12}$ spores + 20 g a.i. insecticide per acre) sprayed alone, in combinations with insecticide and insecticide alone against cotton mealybug (*P. solenopsis*) adults under screen house conditions.

Strain	LT_{50} (days)	95% confidence limit		Chi^2 (df^a=48)
		Lower	Upper	
PDRL526	13.812	10.888	17.852	253.5*
Imidacloprid	13.132	5.319	67.010	979.9*
PDRL526 + Imidacloprid	6.571	3.569	10.355	600.7*

[a] Statistics based on individual cases differ from statistics based on aggregated cases.
[*] Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits.

It appears that the screen house application of the *M. anisopliae* for the management of CMB was carried out for the first time during our studies, since no report on screening of *M. anisopliae* under screen house conditions is available against (*P. solenopsis*) CMB. Although, the *Metarhizium* spp. is widely used as mycoinsecticides against variety of insect pests, even these are popular to control Hemiptera (Faria and Wraight, 2007).

The epf and insecticide synergism showed higher mortalities of insect populations than separate use of insecticide and epf. It suggests that combined use of imidacloprid with the *M. anisopliae* strain helps to start the fungal infection in environment by decreasing insect resistance in tri-trophic levels (Ambethgar, 2009; Roditakis *et al*., 2000; Quintela and McCoy, 1997, 1998; Anderson *et al*., 1989; Hassan and Charnley, 1989). The synergistic treatment promoted the higher yield of cotton seeds/plant. O'Brien (2009), Kan Kang *et al*. (1996), Ownley *et al*. (2010) and Hu and St-Leger (2002) reported that *M. anisopliae* has potential to support plant growth in addition to parasitize the insects. It appears that the present report on the combined application of entomopathogenic fungi and insecticide for the control of *Phenacoccus solenopsis* is very promising, since no prior report is available on synergistic use of entomopathogenic fungi and imidacloprid to CMB. However, similar results were found with other fungal genus, with a combination of imidacloprid and *Beauveria bassiana* strain PDRL1187, that was suitable against mustard aphid (*Lipaphis erysimi*) under field condition (Ujjan and Shahzad, 2014).

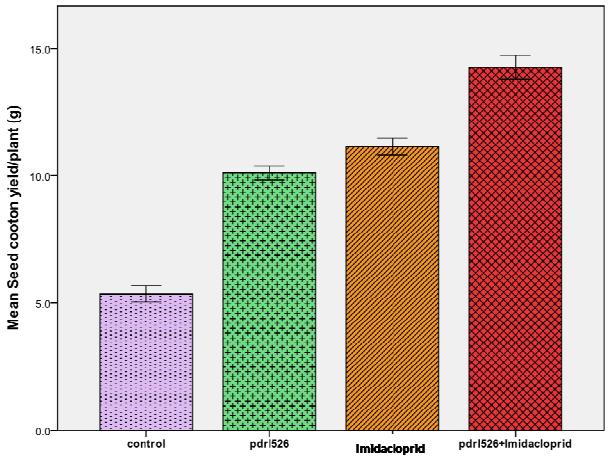

Fig. 2. The yield of seed cotton (g) per plant at field experiments.

Table V.- LT_{50} value of *M. anisopliae* strain PDRL526 applied on cotton mealybug (*P. solenopsis*) adults with single application and in combination of imidacloprid 20 g + $6.30x10^{12}$ spores per acre or single dose of imidacloprid concentration under field conditions.

Strain	LT_{50} (days)	95% Confidence Limit		Chi^2 (df^a=48)
		Lower	Upper	
PDRL526	19.610	15.779	25.803	136.2(22)*
Imidacloprid	14.735	10.058	24.280	296.1(23)*
PDRL526 + Imidacloprid	8.447	6.432	10.870	188.2(23)*

[a] Statistics based on individual cases differ from statistics based on aggregated cases.
[*] Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits.

Field bioassays

The field application of epf showed a comparative decrease in virulence (LT_{50} 19.6 days) as compare to less stressed conditions of screen house (Table V). Although the cotton yield and insect mortality data suggested the strain was efficient at field. When the insecticide applied with the combination of the fungal spores the mortality synergized *i.e*. decreased LT_{50} value (8.4 days) and seed cotton yield (14.5 g/plant) increased (Table V, Fig. 2). The visual evidences of died insects also confirmed the mortality of the insect population in field, when the cadavers incubated on mycological medium (PDA), the *M. anisopliae* growth assured the hypothesis about the virulence of the strain (Fig.3). While the strain efficiency was multiplied to check the CMB population and seed cotton yield, when applied with the insecticide.

The single application of the strain PDRL526 at field condition showed a bit reduction in virulence as compared to the screen house condition. The same results were also reported by Nagrare *et al*. (2011). However, the strains showed protective virulence in field (Table V). The imidacloprid 20 g *a.i.* per acre sustained virulence and caused insect mortality 50 to 60% after 30 days of application, same higher toxicity is reported by Lysandrou *et al*. (2012), when they used imidacloprid @ 125 g *a.i.* per hectare which caused 100% mortality. Suresh *et al*. (2010) reported, imidacloprid 20 g *a.i.* per acre wiped out the CMB population after 3 days of application. Dhawan *et al*. (2009) reported imidacloprid 200SL @ 900 ml per hectare (aprx. 72 g per acre) reduced 81% CMB population at field conditions. Tanwar *et al*. (2007) recommended imidacloprid @ 20 g per acre. The combined application of imidacloprid and epf strains showed synergism against CMB populations.

The strain under present study increased plant yield in cottonseed under field conditions as compared to control plants (Fig. 2). The strain in combined applications with imidacloprid insecticide synergized in insect mortality as well as in cottonseed production (Fig. 2, Table V). Some strains of *M. anisopliae* reported to promote plant growth in addition to insect control (O'Brien, 2009; Kan Kang *et al*., 1996; Ownley *et al*., 2010; Hu and St-Leger, 2002), which suggests the PDRL526 strain has new dimensions for these studies.

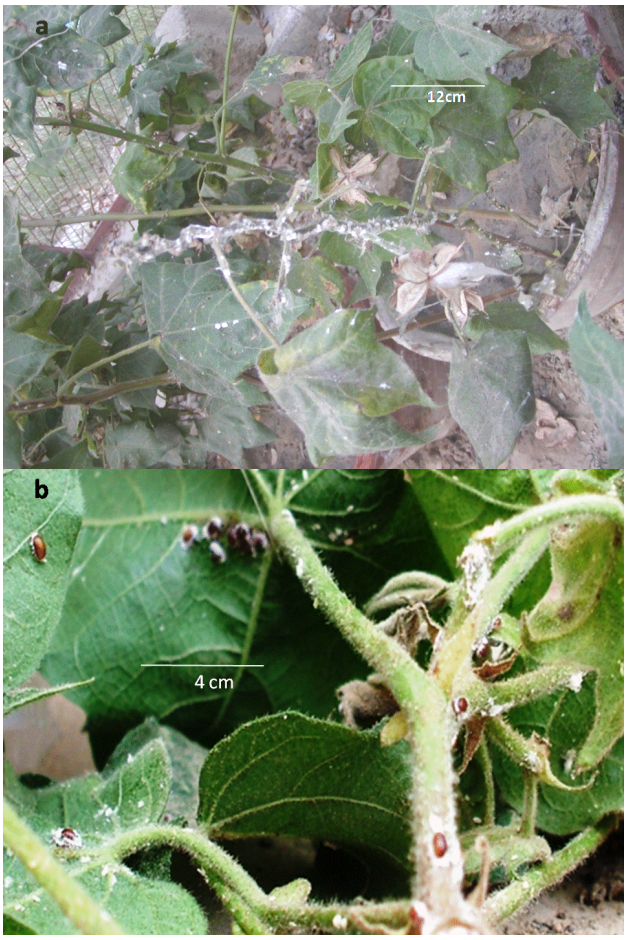

Fig. 3. a, CMB adults' healthy population on artificially infested cotton plant after the spray of spore free (control) suspension at screen house conditions; b, Dead cotton mealybug (*Phenacoccus solenopsis*) adults on artificially infested cotton plants after the bioassays of strains *M. anisopliae* PDRL526 at field conditions after 22 days.

It is, therefore suggested that the strain PDRL526 *M. anisopliae* has sustainable mycoinsecticidal attributes, and it can be utilized against cotton mealybug.

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, that is

gratefully acknowledged.

REFERENCES

ABBOTT, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. econ. Ent.*, **18:** 265-267.

AHSAN, M.T., 2007. *Bioecology and population studies through life table technique of some sucking pests of cotton i.e. cotton leafhopper* Amrasca devastans *(distant) and cotton scale/mealybug* Phenacoccus *(sp.) and their recent control strategies*. Ph.D. dissertation, University of Karachi, Pakistan.

ALIZADEH, A., SAMIH, M.A., KHEZRI, M. AND RISEH, R.S., 2007. Compatibility of *Beauveria bassiana* (Bals.) Vuill. with several pesticides. *Int. J. agric. Biol.*, **9:** 31-34.

AMBETHGAR, V., 2009. Potential of entomopathogenic fungi in insecticide resistance management (IRM): A review. *J. Biopest.*, **2:** 177-193.

ANDERSON, T.E., HAJEK, A.E., ROBERTS, D.W., PREISLER, H.K. AND ROBERTSON, J.L., 1989. Colorado potato beetle (Coleoptera: Chrysomelidae): effects of combinations of *Beauveria bassiana* with insecticides. *J. econ. Ent.*, **82:** 83-89.

ANONYMOUS, 2006. *Economic survey of Pakistan.* Government of Pakistan, Ministry of Food, Agriculture and Livestock. Islamabad..

ANONYMOUS, 2008a. *Emerging pests in North Indian cotton.* NCIPM, New Delhi and CICR, Regional Station, Sirsa. Available on-line at http://www.ncipm.org.in/Mealybugs/ EmergingPest.doc

ANONYMOUS, 2008b. Mealybug: a new threat to cotton production in Pakistan and India. *The ICAC Recorder, Tech. Inf. Sect.*, **26:** 15-19.

ANONYMOUS, 2008c. *Pakistan statistical year book. Ch. 2.* Federal Bureau of Statistics. Agriculture. Statistics Division, Federal Bureau of Statistics, Ministry of Economic Affairs and Statistics, Govt. of Pakistan. Available on-line at http://www.statpak.gov.pk/depts/fbs/publications/yearbook2008/yearbook2008.htm.

ANONYMOUS, 2010. *Development, validation, utilization and / or commercialization of biopesticides, bioinoculants and disease diagnostic tools*, Technology Commission on Cotton, MM-1, annual report 2009-10, Central Institute for Cotton Research Nagpur, India. http://tmc.cicr.org.in/pdf/intro-09-10.pdf.

BANU, J.G., SURULIVELU, T., AMUTHA, M. AND GOPALAKRISHNAN, N., 2010. Laboratory evaluation of insecticides and biopesticides against *Phenococcus solenopsis* and *Paracoccus marginatus* infesting cotton. *J. Biopestic.*, **3:** 343-346.

BARNETT, H.L. AND HUNTER, B.B., 1988. *Illustrated genera of imperfect fungi.* ADS.

BRAND, D., RODRIGUES, C., VANDENBERGHE, L.P.S., MEDEIROS, A.B.P., FERNÁNDEZ, D.E.R., HALFEN, G.E., SILVA, A.L.L., COSTA, J.L., SOCCOL, V.T. AND SOCCOL, C.R., 2012. Fungi application as biological control agents in agriculture and animal husbandry. In: *Biocontrol: Management, processes and challenges* (eds. S.K. Brar), Nova Science Publishers, Inc. Hauppauge, EUA. pp. 171-193.

BUTT, T.M., CARRECK, N.L., IBRAHIM, L. AND WILLIAMS, I.H., 1998. Honeybee mediated infection of pollen beetle (*Meligethes* spp.) by the insect-pathogenic fungus, *Metarhizium anisopliae*. *Biocont. Sci. Technol.*, **8:** 533-538.

DHAWAN, A.K., SINGH, K. AND SINGH, R., 2009. Evaluation of different chemicals for the management of mealybug *Phenacoccus solenopsis* Tinsley on Bt cotton. *J. Cotton Res. Dev*., **23:** 289-294.

DOMSCH, K.H., GAMS, W. AND ANDERSON, T.H., 1980. *Compendium of soil fungi*. Vol. 1 & 2. Academic Press (London) Ltd.

FARIA, M.R.D. AND WRAIGHT, S.P., 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol. Contr.*, **43:** 237-256.

FUCHS, T.W., STEWART, J.W., MINZENMAYER, R. AND ROSE, M., 1991. First record of *Phenacoccus solenopsis* Tinsley in cultivated cotton in the United States. *The Southw. Entomol.*, **16**: 215-221

GOETTEL, M.S. AND INGLIS, G.D., 1997. Fungi: Hyphomycetes. In: *Manual of techniques in insect pathology* (Ed. L.A. Lacey), Academic Press, London. pp. 5- 3.

HALLSWORTH, J. E. AND MAGAN, N., 1994. Effects of KCl concentration on accumulation of acyclic sugar alcohols and trehalose in conidia of three entomopathogenic fungi. *Lett. appl. Microbiol.*, **18:** 8-11.

HASSAN, A.E.M. AND CHARNLEY, A.K., 1989. Ultrastructural study of the penetration by *Metarhizium anisopliae* through Dimilin-affected cuticle of *Manduca sexta*. *J. Inverteb. Pathol.*, **54:** 117-124.

HENDERSON, C.F. AND TILTON, W.W., 1955. Tests with acaricides against the brown wheat mite. *J. econ. Ent.*, **48:** 157-161.

HU, G. AND ST-LEGER, R.J.S., 2002. Field studies using a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent. *Appl. environ. Microbiol.*, **68:** 6383-6387.

HUMBER, R.A., 2012. Identification of entomopathogenic fungi. In: *Manual of techniques in invertebrate pathology* (ed. L.A. Lacey), Academic Press, London, UK. pp. 151-187.

INGLIS, G.D., JOHNSON, D.L., CHENG, K.J. AND GOETTEL, M.S., 1997. Use of pathogen combinations

to overcome the constraints of temperature on entomopathogenic hyphomycetes against grasshoppers. *Biol. Contr.*, **8:** 143-152.

JAMES, R.R. AND ELZEN, G.W., 2001. Antagonism between *Beauveria bassiana* and Imidacloprid when combined for *Bemisia argentifolii* (Homoptera: Aleyrodidae) control. *J. econ. Ent.*, **94:** 357-61.

KAKAKHEL, I., 2007. *Mealybug attack affects cotton crop on 150,000 acres*. http://www.dailytimes.com.pk/default News story 23-8-2007, 54 p.

KAN KANG, C.S., GOO, B.Y., GYU, L.D. AND HEON, K.Y., 1996. Antifungal activities of *Metarhizium anisopliae* against *Fusarium oxysporum*, *Botrytis cinerea* and *Alternaria solani*. *Korean J. Mycol.*, **24:** 49-55.

KIM, J.J. AND KIM, K.C., 2008. Selection of a highly virulent isolate of *Lecanicillium attenuatum* against cotton aphid. *J. Asia-Pacif. Ent.*, **11:** 1-4.

KUMAR, R., NITHARWAL, M., CHAUHAN, R., PAL V. AND KRANTHI, K.R., 2012. Evaluation of ecofriendly control methods for management of Mealybug, *Phenacoccus solenopsis* Tinsley in cotton. *J. Ent.*, **9:** 32-40.

LORD, J.C. 2005. From Metchnikoff to Monsanto and beyond: The path of microbial control. *J. Inverteb. Pathol.*, **89:** 19-29.

LYSANDROU, M., AHMAD AND LONGHURST, C., 2012. Management of mealybug, *Phenacoccus solenopsis* Tinsley in cotton with a new sap feeding insecticide "sulfoxaflor". *J. Agric. Res.*, **50:** 4.

MAKADIA, R.R., KABARIA, B.B., JETHVA, D.M. AND VIRANI, V.R., 2009. Bio-efficacy and cumulative effect of *Verticillium lecanii* against *Maconellicoccus hirsutus* (Green) on custard apple. *Agric. Sci. Dig.*, **29:** 300-302.

METSCHNIKOFF, V.E., 1884. Ueber eine Sprosspilzkrankheit der Daphnien. Beitrag zur Lehre über den Kampf der Phagocyten gegen Krankheitserreger. *Virchows Archiv.*, **96:** 177-195.

NAGRARE, V.S., KRANTHI, S., BIRADAR, V.K., ZADE, N.N., SANGODE, V., KAKDE, G., SHUKLA, R.M., SHIVARE, D., KHADI, B.M. AND KRANTHI, K. R., 2008. Widespread infestation of the exotic mealybug species *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae), on cotton in India. *Bull. entomol. Res.*, **99:** 537-541.

NAGRARE, V.S., KRANTHI, S., KUMAR, R., JOTHI, B.D., AMUTHA, M., DESHMUKH, A.J. AND KRANTHI, K.R., 2011. *Compendium of cotton mealybugs*. Central Institute for Cotton Research, India.

NEVES, P.M.O.J. E.H., PAULO, T.T. AND MOINO, JR. A., 2001. Compatibility of entomopathogenic fungi with neonicotinoid insecticides. *Neotrop. En.*, **30:** 263-268.

O'BRIEN, T., 2009. *The saprophytic life of* Metarhizium anisopliae. Ph.D. thesis. Park College, University of Maryland.

OWNLEY, B.H., GWINN, K.D. AND VEGA, F.E., 2010. Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. In: *The ecology of fungal entomopathogens* (ed. H.E. Roy), Springer, Netherlands. pp. 113-128.

PIMENTEL, D., 2009. Environmental and economic costs of the application of pesticides, primarily in the United States. In: *Integrated pest management: innovation-development process*, Springer Netherlands. pp. 89-111.

QUINTELA, E.D. AND MCCOY, C.W., 1997. Enhancing the pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* to larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) with sublethal doses of Imidacloprid. *Environ. Ent.*, **26:** 1173-1182.

QUINTELA, E.D. AND MCCOY, C.W., 1998. Synergistic effect of Imidacloprid and two entomogenous fungi on behaviour and survival of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in soil. *J. econ. Ent*., **91:** 110-122.

RANGEL, D. E., BRAGA, G.U., FLINT, S. D., ANDERSON, A. J. AND ROBERTS., D.W., 2004. Variations in UV-B tolerance and germination speed of *Metarhizium anisopliae* conidia produced on insects and artificial substrates. *J. Inverteb. Path.*, **87:** 77-83.

RODITAKIS, E.I., COUZIN, D., BARLOW, K., FRANKS, N.R. AND CHARNLEY, A. K., 2000. Improving secondary pick up of insect fungal pathogen conidia by manipulating host behaviour. *Ann. appl. Biol.*, **137:** 329-335.

SAMSON, R.A., EVANS, H.C. AND LATGÉ. J.P., 1988. *Atlas of entomopathogenic fungi*. Springer-Verlag.

ST LEGER, R.J., 2008. Studies on adaptations *of Metarhizium anisopliae* to life in the soil. *J. Inverteb. Path.*, **98:** 271-276.

SURESH, S., JOTHIMANI, SIVASUBRMANIAN, R., KARUPPUCHAMY, P., SAMIYAPPAN, P.R. AND JONATHAN, E.I., 2010. Invasive mealybugs of Tamil Nadu and their management. *Karnataka J. agric. Sci.*, **23:** 6-9.

TANWAR, R.K., JEYAKUMAR, P. AND MONGA, D., 2007. *Mealybugs and their management*. *Tech. Bull. NCIPM*, New Delhi (India).19.

THOMAS, M.B. AND JENKINS, N.E., 1997. Effects of temperature on growth of *Metarhizium flavoviride* and virulence to the variegated grasshopper, *Zonocerus variegatus*. *Mycol. Res.*, **101:** 1469-1474.

UJJAN, A.A. AND SHAHZAD, S., 2012. Use of entomopathogenic fungi for the control of mustard aphid (*Lipaphis erysimi*) on canola (*Brassica napus* L.). *Pak. J. Bot*., **44:** 2081-2086.

UJJAN, A.A. AND SHAHZAD, S., 2014. Insecticidal potential of *Beauveria bassiana* strain PDRL1187 and imidacloprid to mustard aphid (*Lipaphis erysimi*) under field conditions. *Pakistan J. Zool.*, **46:** 1277-1281.

VESTERGAARD, S., GILLIESPIE, A.T., BUTT, T.M., SCHREITER, G. AND EILENBERG, J., 1995. Pathogenicity of the hyphomycetes fungi *Verticillium lecanii* and *Metarhizium anisopliae* to the western flower thrips, *Frankliniella occidentalis*. *Biocontr. Sci. Tech*., **5:** 185-192.

WATSON, G.W. AND CHANDLER, L.R., 2000. *Identification of mealybugs important in the Caribbean region*. CABI Bioscience Egham, Surrey, UK.

ZAKA, S.M., SAEED, S., BUKHARI, S.A. AND BAKSH, E., 2006. Mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae): a novel pest of cotton in Pakistan. In: *Proceedings of 34 Pakistan (SAARC) Countries Science Conference*, p. 32. University of Veterinary and Animal Sciences, Lahore, Pakistan, 2006.

(Received 14 September 2014, revised 11 November 2014)