



# Pathogenicity of Different Isolates of Entomopathogenic Fungi on Cotton Mealybug, *Phenacoccus solenopsis* Tinsley

Madiha Nawaz and Shoaib Freed\*

Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan, Pakistan

## ABSTRACT

Cotton mealybug, *Phenacoccus solenopsis* Tinsley is a destructive pest of cotton, ornamental plants and many other crops due to its polyphagous nature. A study was conducted to check the efficacy of different local isolates of entomopathogenic fungi on 2<sup>nd</sup> nymphal instar of *P. solenopsis* under laboratory conditions by immersion method. Three entomopathogenic fungi; *Beauveria bassiana* (isolates Bb-01, Bb-08), *Metarhizium anisopliae* (isolates Ma-11.1, Ma-2.1) and *Isaria fumosorosea* (isolates If-2.3, If-02) showed percent mortalities of (61.0%, 85.0%), (78.0%, 56.0%) and (52.0%, 54.0%) with LC<sub>50</sub> values of (4.25×10<sup>8</sup>, 2.54×10<sup>8</sup> spores/ml), (3.26×10<sup>8</sup>, 5.08×10<sup>8</sup> spores/ml) and (6.22×10<sup>8</sup>, 7.20×10<sup>8</sup> spores/ml) with LT<sub>50</sub> (6.43, 4.80), (5.43, 6.74) and (6.66, 6.69) days at highest concentrations. Amongst all isolates, *B. bassiana* isolate Bb-08 was highly efficient against cotton mealybug with highest percent mortality and lowest LC<sub>50</sub> and LT<sub>50</sub> values. The study showed that Bb-08 can be used in the IPM of *P. solenopsis*.

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MN performed experiments, analysis of data and manuscript writing. SF provided technical assistance, supervision and manuscript writing.

## Key words

Entomopathogenic fungi, Cotton mealy bug, IPM, Pathogenicity

## INTRODUCTION

Cotton, *Gossypium hirsutum* also famous as white gold is the primary cash crop of Pakistan and it contributes approximately 1.5% of GDP. Cotton crop is attacked by many sucking and chewing insect pests (Saeed *et al.*, 2007) and that is the reason of about 20-40% loss per annum (Ahmad, 1999). Cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has a large ecological pattern (Fuchs *et al.*, 1991; Williams and Granara de Willink, 1992). The cotton mealybug, due to its polyphagous feeding behavior, is a severe insect pest worldwide including Taiwan, Thailand, India and Pakistan (Yousuf and Tayyib, 2007; Hodgson *et al.*, 2008; Abbas *et al.*, 2010). From the Eastern region of Sri Lanka, recent information due to the attack of cotton mealybug is originated (Prishanthini and Vinobaba, 2009) where it is found on vegetables, weeds and ornamental plants and in China, on China rose plants (Wang *et al.*, 2009; Wu and Zhang, 2009). During the year 2005, for the first time in Pakistan (South Asia) this insect pest was reported and in Pakistan (Anonymous, 2008a) and India (Anonymous, 2008b; Nagrare *et al.*, 2008) it has become a widespread pest causing severe damage to *Gossypium* fields

in Punjab and Sindh Provinces (Anonymous, 2006, 2008c; Zaka *et al.*, 2006; Kakakhel, 2007).

Large populations of mealybugs cause general weakening, yellowing and malformation of leaves and defoliation, dropping of fruits and death of susceptible plants if unable to control. Indirectly, it may also damage plants by serving as vectors of plant diseases. Moreover, the honeydew excreted by the mealybugs causes growth of sooty moulds (Saeed *et al.*, 2007), and other secondary infections that decrease photosynthesis and reduces the marketability of plant products. The feeding by mealybugs influences the growing points resulting in smaller fruit or flowers, which eventually decreases seed production (Afzal *et al.*, 2014).

A large number of synthetic pesticides belonging to carbamate, organophosphate, pyrethroid and new chemistry groups are being applied for the management of this insect pest. On the other hand, the cryptic habit and waxy body causes hindrance in the efficient control of *P. solenopsis* with conventional synthetic insecticides. Chemicals provide only short term control, repeated applications and due to injudicious use of these synthetic insecticides, resistance has become the main issue (Ramakrishnan *et al.*, 1984).

Due to drawbacks of synthetic chemical pesticides, it is necessary to develop an alternative tactic such as biological control, which is safe and eco-friendly. Biological control involving insect pathogens, predators

\* Corresponding author: [sfareed@bzu.edu.pk](mailto:sfareed@bzu.edu.pk)  
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and parasitoids, have effectively suppressed mealybugs of majority of the crops, e.g., *Phenacoccus manihoti* (Herren and Neuenschwander, 1991), *Maconellicoccus hirsutus* (Kairo *et al.*, 2000) and *Planococcus citri* (Singh, 2004).

Amongst the biocontrol agents, entomopathogenic fungi serve as mycoinsecticide (Faria and Wraight, 2007) and the use of different entomopathogenic microorganisms are gaining importance due to their target specificity and the environment safety. The pest control scenario primarily of insect pathogenic fungi including, *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* (Marannino *et al.*, 2006; Kaaya and Munyinyi, 1995) have been proved beyond doubt over the decades. Insect pathogenic fungi are the component of integrated control strategies for various insect pests of number of economic cash crops. These fungi are easy to cultivate for mass production and all over the world these microbes are being commercially prepared and effectively used in green houses and field conditions. Another important fact to be considered in favour of these fungi is that, to date there has no report of developing resistance. The present study was conducted to check the efficiency of local isolates of different insect pathogenic fungi against *P. solenopsis* under laboratory conditions.

## MATERIALS AND METHODS

### *Collection and rearing of P. solenopsis*

The population of mealybug, *P. solenopsis* was collected from the cotton field of Bahauddin Zakariya University, Multan. Mealybugs were reared on fresh leaves of China rose, *Hibiscus Rosa sinensis* the preferred ornamental host plant of the mealybug. In order to develop the culture of mealybug, stems of the host plants attacked with adult females were brought to the Laboratory. Insects were separated and inoculated on China rose plants and reared in the lab. The female *P. solenopsis* settled on host plants leaves and twigs, started egg laying after 2 days. The newly hatched crawlers emerged out and started feeding on the China rose leaves which were not exposed to any insecticide applications previously and free from the infestation of *P. solenopsis*. Leaves and twigs were washed with tap water, dried with tissue paper and utilized as food source. The crawlers were placed on China rose leaves with fine camel hair brush. The culture was placed in plastic jars (13×22 cm). Fresh leaves were provided after every 1-2 days under lab. conditions at  $27 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$  RH with a 14:10 h light: dark photoperiod.

### *Propagation of media and culturing of insect pathogenic fungi*

Six different isolates of entomopathogenic fungi

were used to check the toxicity of entomopathogenic; *M. anisopliae* (Ma-11.1 and Ma-2.1), *I. fumosorosea* (If-02 and If-2.3), *B. bassiana* (Bb-01 and Bb-08). Potato dextrose agar (PDA) (potato 200g, glucose 20g, agar 20g and 1000ml water) was freshly prepared by means of distilled water and commercial ingredients. This media was transferred into the petri dishes which were introduced with the spores of different insect pathogenic fungi. Later, after inoculation, all petri dishes were incubated at  $25^\circ\text{C}$  for 14 days and after incubation, the spores were harvested solution in 0.05% Tween 80. The spore's concentration was determined by hemocytometer and afterwards, required concentrations *i.e.*,  $2 \times 10^8$  to  $7 \times 10^8$  spores/ml of each isolate was made by serial dilution. On the PDA plates (9 cm diameter), the spores from slant culture were inoculated which were placed for 14 days at  $25^\circ\text{C}$  in darkness at 70-75% RH for more propagation. Fungal spores were applied to the insects or stored at  $4^\circ\text{C}$  until utilized for bioassay of insect after 14 days of fungal growth.

### *Experimental method and bioassay*

The experiment was conducted under completely randomized design (CRD) with six concentrations including control for each fungal treatment, while each concentration was replicated five times. The efficiency of different entomopathogenic fungi was assessed by immersion method on 2nd instar nymphs of *P. solenopsis*. 750 nymphs of *P. solenopsis* from the laboratory reared culture were exposed with the suspension of all fungal concentrations ( $2 \times 10^8$  -  $7 \times 10^8$  spores/ml) with 25 individuals per replication. Nymphs of *P. solenopsis* were individually immersed in the concentration for least 8-10 sec. Treated insects were placed on the tissue paper to soak up the excess moisture, then transferred into petri dishes and provided with China rose leaves, while in the control treatment, nymphs were treated with 0.05% Tween 80 solution.

Mortality data was recorded on daily basis for continuous 7 days at 24 h interval. Dead nymphs were collected daily and placed in sterile Petri dishes containing damp filter paper. Morality was taken into account for those nymphs which sporulation was visible.

### *Data analysis*

Percent mortality was calculated on each day. The mortality was corrected where necessary by Abbot's formula (Abbot, 1925).  $LC_{50}$  and  $LT_{50}$  values of each isolate were calculated for nymphs by using probit analysis (Finney and Stevens, 1948). Percent mortality was analyzed and compared by LSD test by using Statistics 8.1 statistical software.

## RESULTS

### *Pathogenicity of entomopathogenic fungi against P. solenopsis*

Six isolates of entomopathogenic fungi were found pathogenic to cotton mealybug. Significantly different mortalities were obtained at each conidial concentration tested. The highest mortality was found at concentration  $7 \times 10^8$  conidia/ml in all the four isolates against second instar of mealybug (Table I). The entomopathogenic fungi (isolate Bb-08) was found to be more efficient causing highest mortality of second instar (85.0%) at concentration of  $7 \times 10^8$  conidia/ml. Higher nymphal mortality with increase in spore concentration of entomopathogenic fungi shows that their efficiency was in proportion to the concentration of spores.

### *Concentration and time mortality response*

Tables I and II indicate median lethal concentration

( $LC_{50}$  conidia/ml) and median lethal time ( $LT_{50}$  days), respectively for all entomopathogenic fungi against second instar nymphs of mealybug. The  $LC_{50}$  ( $2.54 \times 10^8$  spores/ml) was found to be lowest for isolate Bb-08 against 2<sup>nd</sup> instar. Similarly, the isolate Bb-08 also showed lowest  $LT_{50}$  values (4.80 days) in respect of second instar of *P. solenopsis*.

### *Accumulative percentage mortality*

Accumulative percentage mortality of isolates of insect pathogenic fungi, *B. bassiana* (Bb-08 and Bb 01), *I. fumosorosea* (If-02, If 2.3) and *M. anisopliae* (Ma-2.1 and Ma-11.1) on mealybug are shown in Figure 1. Percent mortality caused by isolate Bb-08 range from 45.0 to 85.0%, isolate Bb-01 (34.0 to 60.0%), isolate If-02 (32.0 to 54.0%), isolate If-2.3 (32.0 to 52.0%), isolate Ma-2.1 (38.0 to 56.0%) and isolate Ma-11.1(36.0 to 78.0%), respectively over a period of 7 days at the concentrations from  $2 \times 10^8$  to  $7 \times 10^8$  spores/ml.

**Table I.**  $LC_{50}$  (spores/ml) values of different isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea*.

Fungi	Isolates	$LC_{50}$ (spores/ml)	FL <sup>a</sup>	Slope	Chi	n <sup>b</sup>
<i>B. bassiana</i>	Bb-01 Bb-08	$4.25 \times 10^8$	$3.56 \times 10^8$ - $5.08 \times 10^8$	$1.31 \pm 0.25$	0.15	750
		$2.54 \times 10^8$	$1.98 \times 10^8$ - $2.97 \times 10^8$	$1.71 \pm 0.26$	6.49	750
<i>M. anisopliae</i>	Ma-11.1	$3.26 \times 10^8$	$2.81 \times 10^8$ - $3.66 \times 10^8$	$1.97 \pm 0.26$	2.51	750
		$5.08 \times 10^8$	$3.97 \times 10^8$ - $7.79 \times 10^8$	$0.88 \pm 0.25$	0.99	750
<i>I. fumosorosea</i>	If-02	$7.20 \times 10^8$	$5.55 \times 10^8$ - $1.36 \times 10^8$	$0.99 \pm 0.25$	2.60	750
		$6.22 \times 10^8$	$4.96 \times 10^8$ - $9.90 \times 10^8$	$1.03 \pm 0.25$	0.51	750

a: Fudicial limit, b: number of insects treated

**Table II.**  $LT_{50}$  (days) values of different isolates of *B. bassiana*, *M. anisopliae* and *I. fumosorosea* against 2<sup>nd</sup> instar nymphs of *P. solenopsis*.

Fungus	Isolates	Concentrations (spores/ml)	$LT_{50}$	FL <sup>a</sup>	Slope	Chi	n <sup>b</sup>
<i>B. bassiana</i>	Bb-01	$7 \times 10^8$	6.431	5.915-6.993	$3.67 \pm 0.33$	3.42	125
		$6 \times 10^8$	6.425	5.885-7.014	$3.47 \pm 0.31$	0.86	125
		$5 \times 10^8$	6.979	6.324-7.702	$3.51 \pm 0.34$	2.15	125
	Bb-08	$7 \times 10^8$	4.802	4.281-5.386	$4.61 \pm 0.50$	11.2	125
		$6 \times 10^8$	5.674	5.342-6.027	$4.38 \pm 0.36$	3.74	125
		$5 \times 10^8$	6.006	5.598-6.443	$4.01 \pm 0.34$	2.34	125
		$4 \times 10^8$	6.036	5.634-6.466	$4.13 \pm 0.35$	1.65	125
		$3 \times 10^8$	6.626	6.037-7.274	$3.40 \pm 0.31$	0.28	125
<i>M. anisopliae</i>	Ma-2.1	$7 \times 10^8$	6.747	6.162-7.388	$3.64 \pm 0.34$	1.27	125
		$6 \times 10^8$	6.735	6.087-7.451	$3.16 \pm 0.30$	3.28	125
	Ma-11.1	$7 \times 10^8$	5.436	4.479-6.598	$4.10 \pm 0.58$	15.76	125
		$6 \times 10^8$	6.061	5.682-6.465	$4.47 \pm 0.38$	9.62	125
		$5 \times 10^8$	6.088	5.616-6.601	$3.50 \pm 0.30$	2.64	125
		$4 \times 10^8$	6.953	6.319-7.652	$3.62 \pm 0.35$	0.93	125
<i>I. fumosorosea</i>	If-2.3	$7 \times 10^8$	6.665	6.050-7.343	$3.28 \pm 0.30$	0.69	125
		If-02	$7 \times 10^8$	6.698	6.077-7.382	$3.29 \pm 0.31$	0.95

a: Fudicial limit; b: number of insects treated.

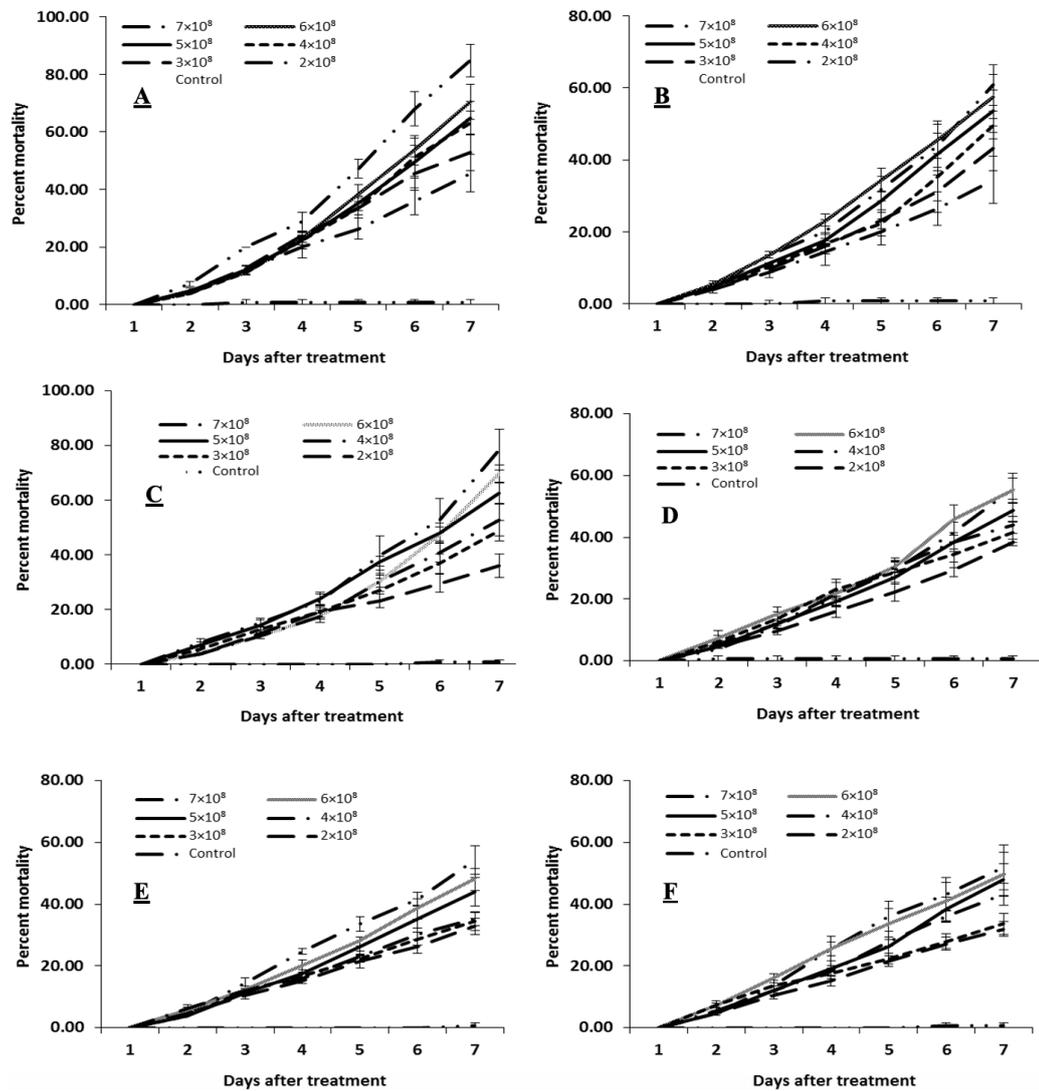


Fig. 1. Percentage mortality of 2<sup>nd</sup> instar nymphs *P. solenopsis* after application of different concentration of (A) *B. bassiana* (Bb 0b), (Bb 01), (c) *M. anisopliae* (Ma 11.1), (D) (Ma 2.1), (E) *I. fumosorosea* (if 02) and (F) (If 2.3). The assessment was done on different days.

## DISCUSSION

Information is not only needed on the biology and feeding activity of the control agent but also on the most susceptible stage of pest species for the successful initiation of a fungal biocontrol program (Cuthbertson *et al.*, 2003). For the period of fungal infection, the first step prior to penetration is the adhesion of fungi to the host cuticle (Boucias *et al.*, 1984). It was suggested that adhesion occurs at three succeeding phases: (a) adsorption of the propagules of fungi to the surface of cuticle; (b) adhesion of the edge between epicuticle and propagules;

(c) germination and development of fungi at the surface of cuticle in insects, until appressoria are grown to begin the stage of penetration (Fragues, 1984). During penetration of fungus through the cuticle of host, hydrolytic enzymes such as chitinases, lipases and proteases are produced that are suggested to be significant for the beginning of the infectivity process leading to cuticle transposition. The conidia of insect pathogenic fungi are found to produce on cuticle of termites through the penetrating germ tube and develop a systemic infection which eventually eradicates the insect (Milner and Staples, 1996).

During the current research, the spores of fungi were

able to develop and penetrate the exposed nymphs of *P. solenopsis*. It has been reported that temperature and the relative humidity are known to be limiting factors for growth of fungus on insects (Glare and Milner, 1991; Ferron, 1981). High rates of infectivity and a rapid kill of bugs by the hyphomycetous fungi were attained at humidity close to saturation (Silvia and Messias, 1985; Romana and Fergues, 1987; Luz, 1990; Romana, 1992; Luz *et al.*, 1994).

Earlier, it has been reported that pathogenicity of *Verticillium lecanii* and *Aschersonia aleyrodis* to silver leaf whitefly, decreased with developmental stage and the older instars were less vulnerable and adults were rarely contaminated (Gindin *et al.*, 2000; Franssen *et al.*, 1987), while in the case of *Helicoverpa* spp, early stages were found less vulnerable to *Nomuraea rileyi* (Mohamed *et al.*, 1977). The vulnerability of various stages of the *P. solenopsis* to the pathogen and its ability to transmit infection among different stages of development and generations, support the potential of entomopathogenic fungi for biocontrol of cotton mealybug.

Screening of entomopathogenic fungi to determine their effectiveness against *Ceratotheripoides claratris*, *Pseudococcus cryptus*, and *Bemisia tabaci* showed *M. anisopliae* to be more efficient against mealy bug causing 73% mortality. The efficiency of *I. fumosorosea* and *Paecilomyces lilacinus* was not as that of *M. anisopliae* (Panyasiri *et al.*, 2007). It was reported that *Isaria farinosa* caused high mortality, up to 90% at 95% RH and  $1 \times 10^8$  conidia  $\text{ml}^{-1}$ . *I. fumosorosea* caused 87.76% mortality on *Aphelinus asychis* a parasitoid of *Diuraphis noxia*. Additionally, it was highlighted that the mortality was associated with the fungal concentration. Spores concentration is a significant aspect on the pathogenicity of the insect pathogenic fungi (Lacey *et al.*, 1977). The application of *I. farinose* to *Eurygaster integriceps* on pine litter and wheat plants at different concentrations showed that the percent mortality was significantly higher at higher concentration i.e.,  $1 \times 10^8$  conidial concentrations (Parker *et al.*, 2003). An association between the conidial concentration of *I. farinosa* and mortality percentages of *Pissodes punctatus* has also been determined. Our results are parallel to those in which *I. farinosa* caused significant mortality at  $1 \times 10^8$  conidia  $\text{ml}^{-1}$  of *P. citri* ovisacs (Yang *et al.*, 2009).

The current outcomes are according to earlier studies reporting *B. bassiana* to cause highest mortality and *M. anisopliae* to be least efficient than *B. bassiana* in controlling mealybugs (Lacey *et al.*, 2001; Lemawork, 2008). Foliar spray of *V. lecanii* or *B. bassiana* ( $2 \times 10^8$  CFU/ml) @ 5 g / mL per L of water is efficient during high humid months in minimizing the population of

mealybugs (Tanwar *et al.*, 2007). *B. bassiana* @ 5g / L decreased invasion of *P. marginatus* from 90 to 57.78% after exposure under field conditions (Suresh *et al.*, 2010).

## CONCLUSION

In conclusion, the results obtained in these experiments establish the pathogenicity of entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, *I. fumosorosea* on 2<sup>nd</sup> nymphal instar of mealybug as biocontrol agents. All entomopathogenic isolates showed high cumulative mean mortality to second instar mealybugs but *B. bassiana* isolate (Bb-08) caused higher mortality. Moreover, isolate Bb-08 was superior in terms of lower  $\text{LC}_{50}$  and  $\text{LT}_{50}$  values and that makes it ideal candidate for commercial exploitation. However, detailed study of entomopathogenic fungi on cotton mealy bug, *P. solenopsis* should to be undertaken to ascertain its virulence.

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### Statement of conflict of interest

The authors declare there is no conflict of interest.

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