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

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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 2nd 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 LC50 values of (4.25×108, 2.54×108 spores/ml), (3.26×108, 5.08×108 spores/ml) and (6.22×108, 7.20×108 spores/ml) with LT50 (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 LC50 and LT50 values. The study showed that Bb-08 can be used in the IPM of P. solenopsis.

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...
and parasitoids, have effectively suppressed mealybugs of majority of the crops, e.g., Phenacoccus manihoti (Herren and Neuenschwander, 1991), Macoellellicoccus 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 ± 2°C and 60 ± 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°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., 2×10⁸ to 7×10⁸ spores/ml of each isolate was made by serial dilution. On the PDA plates (9 cm diameter), the spores from plant culture were inoculated which were placed for 14 days at 25°C in darkness at 70-75% RH for more propagation. Fungal spores were applied to the insects or stored at 4°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 (2×10⁸ - 7×10⁸ 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). LC₅₀ and LT₉₀ 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 2nd 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*.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Isolates</th>
<th>LC$_{50}$ (spores/ml)</th>
<th>FL$^a$</th>
<th>Slope</th>
<th>Chi</th>
<th>n$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. bassiana</em></td>
<td>Bb-01</td>
<td>$2.54 \times 10^8$</td>
<td>2.56</td>
<td>1.31</td>
<td>0.15</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>Bb-08</td>
<td>$4.25 \times 10^8$</td>
<td>5.08</td>
<td>1.71</td>
<td>6.49</td>
<td>750</td>
</tr>
<tr>
<td><em>M. anisopliae</em></td>
<td>Ma-11.1</td>
<td>$3.26 \times 10^8$</td>
<td>2.81</td>
<td>1.97</td>
<td>2.51</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>Ma-2.1</td>
<td>$5.08 \times 10^8$</td>
<td>3.97</td>
<td>0.88</td>
<td>0.99</td>
<td>750</td>
</tr>
<tr>
<td><em>I. fumosorosea</em></td>
<td>If-02</td>
<td>$7.20 \times 10^8$</td>
<td>5.55</td>
<td>0.99</td>
<td>2.60</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>If-2.3</td>
<td>$6.22 \times 10^8$</td>
<td>4.96</td>
<td>1.03</td>
<td>0.51</td>
<td>750</td>
</tr>
</tbody>
</table>

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 2nd instar nymphs of *P. solenopsis*.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Isolates</th>
<th>Concentrations (spores/ml)</th>
<th>LT$_{50}$</th>
<th>FL$^a$</th>
<th>Slope</th>
<th>Chi</th>
<th>n$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. bassiana</em></td>
<td>Bb-01</td>
<td>$7 \times 10^8$</td>
<td>6.431</td>
<td>3.67</td>
<td>3.42</td>
<td>125</td>
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<tr>
<td></td>
<td></td>
<td>$6 \times 10^8$</td>
<td>6.425</td>
<td>3.47</td>
<td>0.86</td>
<td>125</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>$5 \times 10^8$</td>
<td>6.979</td>
<td>3.51</td>
<td>2.15</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bb-08</td>
<td>$7 \times 10^8$</td>
<td>4.802</td>
<td>4.61</td>
<td>11.2</td>
<td>125</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>$6 \times 10^8$</td>
<td>5.674</td>
<td>4.38</td>
<td>3.74</td>
<td>125</td>
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<tr>
<td></td>
<td></td>
<td>$5 \times 10^8$</td>
<td>6.006</td>
<td>4.01</td>
<td>2.34</td>
<td>125</td>
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<td></td>
<td></td>
<td>$4 \times 10^8$</td>
<td>6.036</td>
<td>4.13</td>
<td>1.65</td>
<td>125</td>
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<tr>
<td></td>
<td></td>
<td>$3 \times 10^8$</td>
<td>6.626</td>
<td>4.30</td>
<td>0.28</td>
<td>125</td>
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<tr>
<td><em>M. anisopliae</em></td>
<td>Ma-2.1</td>
<td>$7 \times 10^8$</td>
<td>6.747</td>
<td>3.64</td>
<td>1.27</td>
<td>125</td>
<td></td>
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<td></td>
<td></td>
<td>$6 \times 10^8$</td>
<td>6.735</td>
<td>3.16</td>
<td>3.28</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ma-11.1</td>
<td>$7 \times 10^8$</td>
<td>5.436</td>
<td>4.10</td>
<td>15.76</td>
<td>125</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>$6 \times 10^8$</td>
<td>6.061</td>
<td>4.47</td>
<td>9.62</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$5 \times 10^8$</td>
<td>6.088</td>
<td>3.50</td>
<td>2.64</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$4 \times 10^8$</td>
<td>6.953</td>
<td>3.62</td>
<td>0.93</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td><em>I. fumosorosea</em></td>
<td>If-2.3</td>
<td>$7 \times 10^8$</td>
<td>6.665</td>
<td>3.28</td>
<td>0.69</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$7 \times 10^8$</td>
<td>6.698</td>
<td>3.29</td>
<td>0.95</td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

a: Fudicial limit; b: number of insects treated.
Fig. 1. Percentage mortality of 2nd instar nymphs *P. solenopsis* after application of different concentrations of (A) *B. bassiana* (Bb 0b), (B) (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 appresoria 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; Fransen 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 *Ceratothripoides claratris*, *Pseudococcus cryptus*, and *Bemisia tabaci* showed that the percent mortality was significantly higher in older instars compared to younger instars (Tanwar et al., 2007). B. bassiana @ 5 g / 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 2nd 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 LC<sub>50</sub> and LT<sub>50</sub> 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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