Efficacy of Entomopathogenic Fungi as Biological Control agent against insect pests of Gossypium hirsutum.

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ABSTRACT
Isolates of *Beauveria bassiana*, *Metarhizium anisoplaie*, *Verticillium lecanii* and *Paeciliomyces lilicuns* from Punjab, Pakistan, were evaluated for their inhibitory and insecticidal efficacy against White fly, House fly, American bollworm, Army worm, Spotted worm, Gray weevil, Jassids, Aphids, Ant and moth. Four strains of *B. bassiana*, two strains of *P. lilicuns* and one strain of *M. anisoplaie* and *V. lecanii* were used. *B.bassiana* (Bb04) exhibited significant mortality percentage against moth, gray weevil, cotton seed bug white fly, American worm, spotted worm, army worm, house fly, ants, jassids and aphids. Among all the strains of entomopathogenic fungi used, *B. bassiana* strains Bb01 revealed least mortality percentage against the targeted insect pests. *V. lecanii* Vl01 strain showed the highest mortality rate against cotton seed bug while it showed least efficiency against spotted worm. *M. anisopolie* Ma01 showed the highest mortality percentage against moth and showed the minimum results against spotted worm. In case of tested strain of *P. lilicuns* strains Pl01 and Pl02 displayed the highest mortality percentage against gray weevil, cotton seed bug and showed the minimum results against spotted worm and moth.

Key words: Entomopathogenic fungi, Pathogenicity, Cotton pests, White fly, American bollworm, Army worm, Gray weevil

INTRODUCTION
Gossypium hirsutum is an integral fiber and cash crop of Pakistan covering almost 2984 thousand hectares of cultivable land. According to economic survey of Pakistan 2012-2013 the annual production of cotton in Pakistan in about 12 million bales but per acre yield of cotton in Pakistan is very low as compared to other major cotton growing countries (Anonymous, 2001 & Bakhsh *et al.,* 2005). Among various factors responsible for low yield of cotton, insect pests are the most important causing 30-40% yield loss (Kannan et al., 2004; Haque, 1991;). About 93 to 145 insect and mite pests attack cotton crop. These include sucking as well as chewing insect pest complex. Among sucking insect pests, dusky cotton bug, *Oxycarenus* species sp. or spp. (Hemiptera: Oxycarenidae) recently has become common widespread pest of economic importance. Both nymphs and adults feed on immature seeds causing multiple types of injuries to the crop including reduction in cotton yield, seed weight and oil contents (Srinivas and Patil, 2004).

Entomopathogenic fungi are an important and widespread component of most terrestrial ecosystems. It seems they are not only in places where there are no victims – insects nor other arthropods. Of course spread of individual species of entomopathogenic fungi are different. However some of them can be found practically throughout the world. An example of such species may be *Beauveria bassiana* which is reported from tropical rainforest (Aung et al., 2008). Despite the fact that both *B. bassiana* and *M. anisopliae* are common everywhere there is known that *B. bassiana* seems to be very sensitive to the disturbance effects of cultivation and thus restricted to natural habitats. The ability of *M. anisopliae* to persist in cultivated soils is well established. Therefore the first is more frequent in forest, and second in arable soils (Quesada-Moraga et al., 2007; Sánchez-Peña et al., 2011). Entomopathogenic fungi are a very heterogeneous group of insect pathogens. It is known nearly 700 species belonging to approximately 100 orders. Although only a few of them have been studied well. Most of them belong to the order *Entomophthorales* of the phylum *Glomeromycota* and to *Hypocreales* of the phylum *Ascomycota* (Hibbett et al., 2007; Sung et al., 2007). Chemical insecticides are commonly used in plant protection. The consequence of this is to increase the resistance of insects to various chemical substances contained in plant protection products. Over 500 arthropod species now show resistance to one or more types of chemicals (Mota-Sanchez et al., 2002). The anamorphic entomopathogenic fungi Beauveria bassiana (Balsamo) Vuillemin and Metarhizium anisopliae (Metschnikoff) Sorokin from the order Hypocreales (Ascomycota) are natural enemies of a wide range of insects and arachnids and both fungi have a cosmopolitan distribution (Roberts and St. Leger, 2004; Rehner, 2005).

MATERIALS AND METHODS

Isolation of entomopathogenic fungi

The infected insect cadavers were collected during survey, brought to the laboratory and pathogens were isolated on Potato Dextrose Agar (PDA). For the isolation of entomopathogenic fungi collected insect samples were surface sterilized with 2 % Sodium hypochlorite (NaOCl), rinsed thrice in plenty of sterilized water and dried by filter papers. The specimens were crushed and inoculated to a culture plate containing potato dextrose agar (PDA) and incubated at 25^0C for 5 days. Plates kept under constant observation for the growth and development of microorganisms. After 5 days of incubation, the organisms were sub-cultured for purification. Slants of each culture were prepared from purified culture and microscopic observations were made. Preliminary identification of fungi was made with the help of the Atlas of entomopathogenic fungi (Samson *et al*., 1988, Lazgeen *et al.,* 2011).

E.F	Strain	Source	Crop	Place of collection
M. anisoplaie	Ma01	Gray weevil	Cotton	Lahore
V. lecanii	Vl01	Moth	Cotton	Lahore
p. lilicuns	Pl01	cotton bug	Cotton	Multan
p. lilicuns	Pl02	Jassid	Rice	Sialkot
B. bassiana	Bb01	Gray weevil	Cotton	Lahore
B. bassiana	Bb02	Gray weevil	Cotton	Multan
B. bassiana	Bb03	cotton bug	Cotton	RYK
B. bassiana	Bb04	cotton bug	Cotton	Sadiqabad

Table1: Total entomopathogenic fungi and their different strains were collected from different area of Punjab.

Maintenance of culture

'A loopful of inocula from subculture plates of isolates were shifted to Potato Dextrose Agar (PDA) slants and sustained as pure cultures. Virulence was revived by passing through an insect host after 5-6 subculturing. For laboratory studies, the fungus was cultured on PDA medium. After complete sporulation, conidia from the medium were harvested by washing them thoroughly with sterilized water containing Tween-80 (0.2%) for immediate use. Spores were also harvested with the help of a small sterile metal spatula. Harvested conidia were air dried under laminar air flow and stored in a small air tight screw cap vials (10 cm with 2.5 cm diameter) in refrigerator at 4^oC. Colony forming units (cfu) were estimated by plating technique. Suspension of spores was made using distilled water with Tween-80 (0.2%) and filtered through a double layered muslin cloth. Spore count was made using a double rolled Neubauer's haemocytometer after necessary serial dilutions under phase contrast microscope. From the stock solution, further dilutions were made to obtain the required concentrations for further studies (Masoud and Bahar, 2012)**.**

Pathogenic activity of entomopathogenic fungi against different insect pests

The insects were collected from the field. The fungal spores were applied on early instar and adults of insects with a sprayer. Three replications were maintained and in each replication 50 larvae/nymph and adults were tested against each entomopathogenic fungi reared in insect culture plates supplemented with leaves on 2% agar gel. The numbers of dead larvae were counted after fifth day of inoculation. Finally, the per cent mortality of each isolate was computed as under (Marek, 2010)

Mortality percentage = number of dead insects/total number of exposed insects x100

Statistical analysis

The data obtained from the laboratory and field experiments were statistically analyzed following standard procedures. Percentage values were transformed to arc sin values while, root transformation ($\sqrt{x}$ + 0.5) was followed for larval or nymphal counts (both total and mycosed) wherever necessary. The data collected were subjected to pooled analysis of variance. Means were separated by DMR (Gomez and Gomez, 1984).

RESULTS

Pathogenicity of entomopathogenic fungi against important pests

The results were presented in fig 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. The strain Bb04 showed the significant mortality percentage against insect moth, gray weevil, cotton seed bug and white fly, American worm, spotted worm, army worm, house fly, ants, jassids and aphids. Among all entomopathogenic fungi, *Beauveria bassiana* strains Bb01 showed least mortality percentage against the listed insects. *V. lecanii* Vl01 strain showed the highest mortality percentage against cotton seed bug and showed minimum results against spotted worm. *M. anisopolie* Ma01 showed the highest mortality percentage against moth and showed the minimum results against spotted worm. In case of tested strain of *paeciliomyces lilicuns* strain Pl01, Pl02 showed the highest mortality percentage against gray weevil, cotton seed bug and showed the minimum results against spotted worm and moth respectively. On the basis of these results strain of *Beauveria Bassiana* Bb04 was best strain of entomopathogenic fungi.

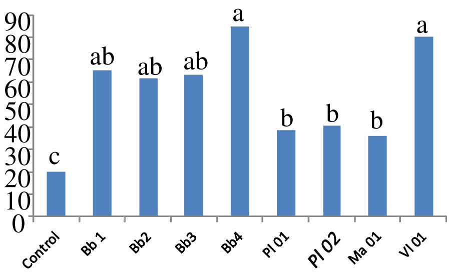

Fig-1 Mortality percentage of spotted worm with different stains of entomopathogenic fungi after 72 hours spore application on insects

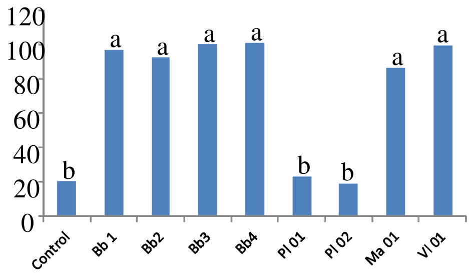

Fig-1 Mortality percentage of Moth with different stains of entomopathogenic fungi after 72 hours spore application on insects

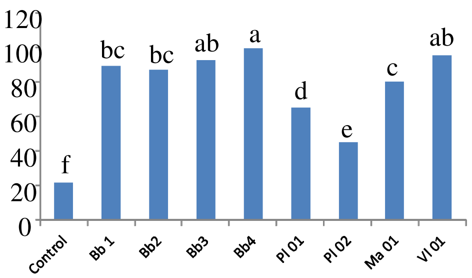

Fig-3 Mortality percentage of aphids with different strains of entomopathogenic fungi after 72 hours spore application on insect.

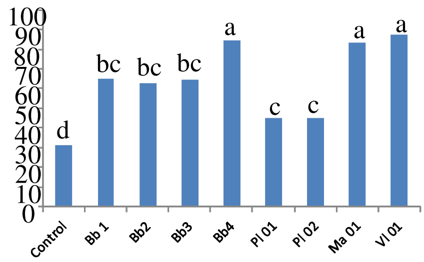

Fig-4 Mortality percentage of American worm with different strains of entomopathogenic fungi after 72 hours spore application on insects.

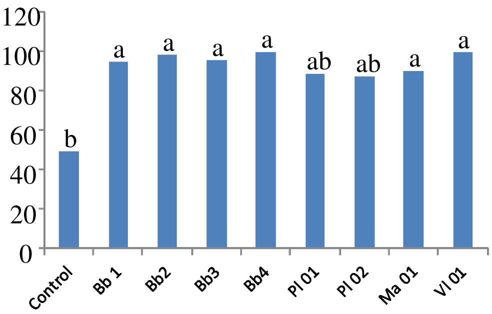

Fig-5 Mortality percentage of Gray weevil with different strains of entomopathogenic fungi after 72 hours spore application on insects.

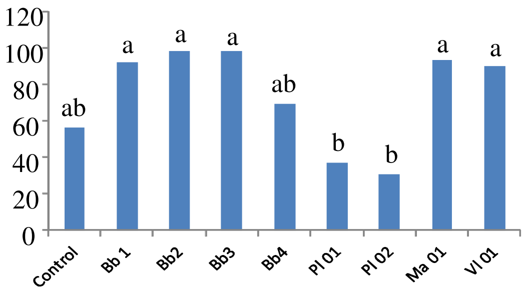

Fig-6 Mortality percentage of white fly with different strains of entomopathogenic fungi after 72 hours spore application on insects.

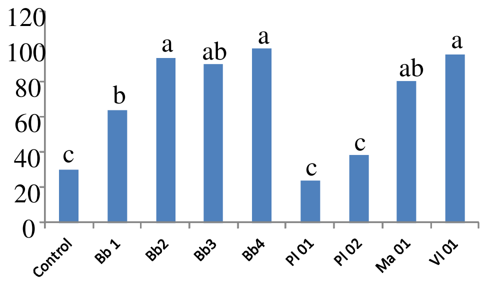

Fig-7 Mortality percentage of jassids with different strains of entomopathogenic fungi after 72 hours spore application on insects

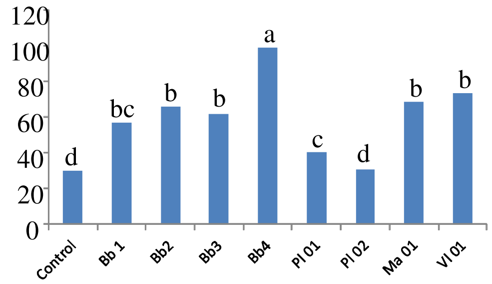

Fig-8 Mortality percentage of army worm with different strains of entomopathogenic fungi after 72 hours spore application on insects

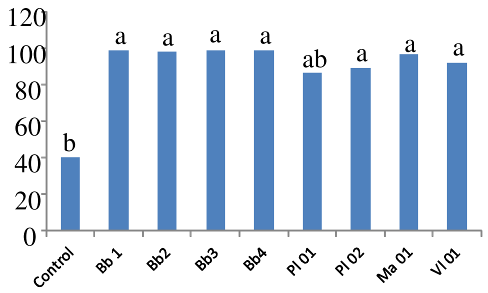

Fig-9 Mortality percentage of cotton seed bug with different strains of entomopathogenic fungi after 72 hours spore application on insects

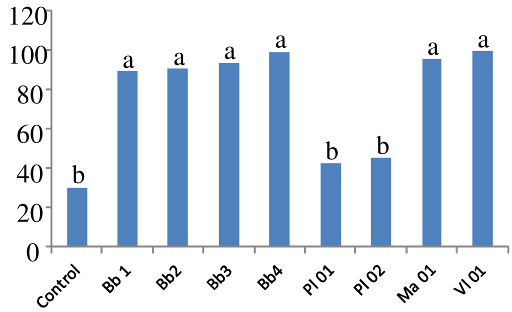

Fig-10 Mortality percentage of house flies with different strains of entomopathogenic fungi after 72 hours spore application on insects

Discussion

The cadavers of susceptible host carry to the laboratory and those supposed to be infected by *B. bassiana, M. anisopliae* and *V. lecanii* were used for separation of the mycopathogen. A total of eight fungi were isolated, decontaminate and recognized based on their colony trait and microscopic assessment. Of the eight, four were identified to be *B. bassiana* one is *M. anisopliae,* one was assigned to *V. lecanii* and two were *paeciliomyces lilicuns*. The individuality of the fungi was later confirmed at fungal culture bank Pakistan. The hyphal and conidial font did not vary between the isolates collected from diverse places in the duration of survey. Though, *B. bassiana* presented considerable deviation in the number of days taken for sporulation, sporulation, spore succumb and time taken to cover up the given diet for mass culturing. Between the different isolates of *B. bassiana,* verification with highest colony diameter but smallest amount spore yield. These results point out changeability between the isolates which need to be appreciate when being used for expansion of efficient bio control and mass culturing.

Pathogenicity of isolates entomopathogenic fungi were experienced against different cotton pests. Four strains of *B. bassiana*, two strains of *P. lilicuns* and one strain of *M. anisoplaie* and *V. lecanii* were used. *B.bassiana* (Bb04) exhibited significant mortality percentage against moth, gray weevil, cotton seed bug white fly, American worm, spotted worm, army worm, house fly, ants, jassids and aphids. Among all the strains of entomopathogenic fungi used, *B. bassiana* strains Bb01 revealed least mortality percentage against the targeted insect pests. *V. lecanii* Vl01 strain showed the highest mortality rate against cotton seed bug while it showed least efficiency against spotted worm. *M. anisopolie* Ma01 showed the highest mortality percentage against moth and showed the minimum results against spotted worm. In case of tested strain of *P. lilicuns* strains Pl01 and Pl02 displayed the highest mortality percentage against gray weevil, cotton seed bug and showed the minimum results against spotted worm and moth(Figure 1-10). These results are in agreement with Ibrahim *et al.,* 2011. They found pathogenicity of the similar entomopathogenic fungi against aphids and white fly. Similar results are also presented by the research work of Jesusa, *et al*., 2010. They found strains of *B. bassiana*, *P. lilicuns* and *M. anisoplaie* as a very effective biological control agent against cactus moth and other pests also.

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