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VIRULENCE OF ENTOMOPATHOGENIC FUNGI ON DIFFERENT LIFE STAGES OF PINK BOLLWORM, *PECTINOPHORA GOSSYPIELLA*

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ABSTRACT

The pink bollworm (*Pectinophora gossypiella*) is one of the most invasive insects globally and a voracious pest of cotton. Chemical pesticides were primarily utilized to manage this insect pest and boost crop productivity, which led to a resistant population and negative environmental and human health repercussions. As a result, more alternative techniques such as biological control, are required. The current study was carried out to assess the effectiveness of several entomopathogenic fungi (EPF) *viz. Beauveria bassiana*, *Metarhizium anisopliae, Verticillium lecanii*, and *Aspergillus flavus* against 2nd instar *P. gossypiela* larvae due to pesticide resistance. Finding revealed that maximum mortality was recorded at highest concentration (1×10^9 spores/ml). *B. bassiana* showed the best result followed by *M, anisopliae*, *V. lecanii* and *Aspergillus flavus* with highest concentration 1×10^9 at 12 post treatment interval with maximum percent mortality (51.62 ± 6.25), (44.27 ± 5.20), (40.39 ± 4.66) and (35.94 ± 3.95) while minimum percent mortality was observed at 3 days of post treatment interval (13.38 ± 1.32), (10.07 ± 1.04), (7.84 ± 0.76) and (7.29 ± 0.86) respectively. EPFs demonstrated their improved capacity to protect cotton crops against *P. gossypiella* over the long term. A viable substitute for pesticides and a useful part of an IPM campaign against *P. gossypiella* is the integration of cultural management techniques with EPFs. EPFs demonstrated the ability to cause the highest mortality rate when it came to pink bollworm.

Keywords: Mortality, virulence, *Pectinophora gossypiella*; *Beauveria bassiana*; *Metarhizium anisopliae*; *Verticillium lecanii* and *Aspergillus flavus*

INTRODUCTION

Pink Bollworm known as *Pectinophora gossypiella* (Lepidoptera: Gelechidae) is one of the major economic pests of cotton all over the world (Tabashnik *et al*., 2000; Naik *et al*., 2020; Nagaraju *et al*., 2024). In Indo-Pak, it is reported first time in 1842 and again in Punjab in 1894, 1917 and 1922 (Attique *et al*., 2004; Jaleel *et al*., 2014). In Pakistan, pink bollworm has become resistant to Bt and conventional varieties of cotton. It has become a worldwide concern due to substantial yield losses of 2.8-61.9% (seed cotton), 2.1-47.1% (oil contents), and 10.7-59.2% (cotton bolls) (Patil, 2003; Chakravarthy *et al*., 2016; Prasad *et al*., 2018; Bhute *et al*., 2023). It feeds on flowers buds, flowers, bolls, seeds with in the boll, and damages lint. It burrows through the lint and, penetrate into immature seeds and feed them. When one seed is destroyed, it moves towards the other seed. Affected bolls become rot and shed, while rest open prematurely (Sarwar, 2017; Shah *et al*., 2020).

To control pink bollworm, different tactics are used including biocontrol, chemical and physical . Traps are used all over the world (Delta traps). Baits made from rubber septa, treated with 1000 µg of lure dispensed in 50 µL of methylene chloride (dichloromethane) are also used (Fite *et al*., 2020; Desneux *et al*., 2022). Conventional insect pest control tactics relied mainly on chemicals. Synthetic insecticides used to manage these pests cause a number of issues, and their leftovers are poisonous to beneficial insects like honey bees and wildlife like birds (Begum *et al*., 2017; Sugumaran *et al*., 2024). These chemical pesticides also injure non-target insects by causing several chemical changes that are detrimental to parasitoids, predators, and other organisms. Both people and domesticated animals are harmed by the chemical pesticides. Entomopathogenic fungi are essential natural regulators of insect populations. According to Jiang *et al*. (2019), insect pests do not readily develop resistance to entomopathogenic fungal infection. Due to pesticide resistance and overuse of insecticides on the crop, cotton pest management has been especially affected. Resistance to pesticides rendered them ineffective, necessitating more frequent applications and resulting in environmental degradation (Kranthi, 2007; Gill and Garg, 2014; Fu *et al*., 2022). Therefore, the current situation requires a health and environmen friendly way to dealing with this hazardous pest. The most efficient and environmentally friendly bioagents that enter the host through their cuticle are the entomopathogen fungi (Niu *et al*., 2019). These fungi are effective against a variety of insect pests, and they have a wide host range because of their antagonistic natures and non-specific effects (Ong and Vandermeer, 2014; Ahmad *et al.*, 2022; Sarkar *et al*., 2024).

Entomopathogenic fungi namely *B. bassiana* and *M. anisopliae* are pathogenic to a variety of agricultural insect pests (Rodrigues *et al*., 2016). According to Sarkar *et al*. (2024), *M. anisopliae* and *B. bassiana* have demonstrated the ability to suppress a number of insect pests, including the cotton leaf warm *Spodoptera littoralis*, and the cotton bollworm *Helicoverpa armigera*. It has been demonstrated that entomopathogenic fungi kill *P. gossypiella* at various stages (Abd-ElAzeem *et al*., 2024; Sarkar *et al*., 2024). EPF are considered cosmopolitan related with insects and plant feeding mites (Sharma *et al*., 2023). There are more than 700 species and 90 genera of fungi among which *Metarhizum, Verticilium, Beauveria, Isaria* and *Purpureocillium* are mostly used as bioagents to control the agricultural pests (Rizwan *et al*., 2019; Gohel *et al*., 2022; Abd-El Azeem *et al*., 2024; Sarkar *et al*., 2024). The present study aimed to check the efficacy of various entomopathogens fungi (*Beauveria bassiana, Verticilium lecanii, Aspergillus flavus* and *Metarhizum anisopliae*) on various biological parameters of pink bollworm under laboratory conditions.

MATERIALS AND METHODS

Rearing of pink bollworm

In a lab, pink bollworm (PBW) culture was maintained. Its larvae were taken from infested fields and brought to the IPM Laboratory at the Department of Entomology, University of the Punjab Lahore, Pakistan. The culture was maintained at 27.2°C and 70% RH in a laboratory setting (Abd El-Hafez *et al*., 1982). When moths appeared, the freshly hatched larvae of the pink bollworm were placed in the previously specified settings, sexed, and fed a 10% sucrose solution. They were then grown on an artificial diet. In a muslin-covered glass cage, females were permitted to deposit their eggs, which were subsequently counted when the cage was changed every other day.

Culture conditions

Four EPF strains *viz., Aspergillus flavus, Verticillium lecanii, Beauveria bassiana* and *Metarhizium anisopliae* provided by the USDA, USA, were grown on PDA plates in 9 cm-diameter Petri dishes and incubated for 15 to 20 days at 27°C. A sterile glass slide was used to gently scrape the surface of a culture that was 15-20 days old in order to extract the spores. The spores were suspended in 0.1% Tween-20 in distilled water. Five spore suspension concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 spores mL^{-1}) were prepared for each isolate by shaking the mixture for 10 min using a magnetic shaker.

Treatment of *P. gossypiella* larvae

For 10 s, 30 *P. gossypiella* larvae in their second instar were submerged in conidial suspensions of EPF (*B. bassiana, V. lecanii, M. anisopliae* and *A. flavus*) in concentrations ranging of 1×10^5, 1×10^6, 1×10^7, 1×10^8, 1×10^9 spores mL^{-1}. The larvae were then placed separately in dishes and air dried for 10 min in sterile Petri dishes before receiving artificial feed to study mortality. The mortality of the larvae was recorded and converted into percent mortality. The mortality rate was calculated at intervals of 5, 7, and 10 days.

Statistical analysis

To ascertain the impacts of both individual and interacted application of variables, the data was analyzed using statistical program the Statistix® (version 8.1) and analysis of variance (ANOVA) in CRD. Mean values at $P \leq 0.05$ were compared using Tukey's HSD test for mean separation.

RESULTS

Thirty 2nd instars larvae of *P. gossypiella* treated with different EPF *B. bassiana, M. anispolea*, *V. lecanii* and *Aspergilus* sp. each showed significant results at different exposure and different concentrations. At 12 day after incubation, *B. bassiana* larvae showed the highest mortality (51.62%), while the lowest mortality by *A. flavus* was 35.94%. On the other hand, at the highest concentration (1×10^9) maximum mortality recorded was 46.10%by *B. bassiana*, while *Aspergillus* caused lowest mortality 36.92%. Mortality of all the treatments was compared to control whose mortality rate was less than 5%. Effects of the lowest and the highest concentration of all the four EPF were recorded significant. Maximum mortality due to *B. bassiana* was 46.10%, followed by *M. anisopliae*, *V. lecanii*, and *A. flavus* 44.42%, 40.26%, 36.92%, respectively at the highest concentration of 1×10^9, while minimum mortality was recorded at the lowest concentration of 1×10^5 was in range of 20.26%, 21.92%, 17.66%, and 13.59%, respectively, as shown in Table 1.

Table 1. Percentage mortality rate (mean ± SE) of *Pectinophora gossypiella* larvae 2nd instar treated with varying EPF strain concentrations.

Concentrations (Spores/ml)	**Fungi**			
	Beauveria bassiana	***Metarhizium anisopliae***	***Verticillium lecanii***	***Aspergillus flavus***
1×10^5	20.26 ± 3.14^E	21.92 ± 3.24^E	17.66 ± 2.91^E	13.59 ± 2.79^E
1×10^6	25.26 ± 4.16^D	25.26 ± 3.69^D	21.09 ± 3.54^D	17.66 ± 3.16^D
1×10^7	31.93 ± 4.79^C	29.42 ± 4.20^C	26.09 ± 4.20^C	23.59 ± 3.86^C
1×10^8	38.60 ± 6.65^B	38.59 ± 5.65^B	33.59 ± 5.21^B	30.26 ± 4.53^B
1×10^9	46.10 ± 7.91^A	44.42 ± 6.44^A	40.26 ± 6.11^A	36.92 ± 5.01^A
Control	4.435 ± 0.49^F	4.42 ± 0.47^F	4.42 ± 0.47^F	4.42 ± 0.47^F

Similar letters are not significant with each other in a column according to DMRT at $p < 0.05$.

Table 2. Efficacy of different concentration of EPF strains against 2nd instar larvae of pink bollworm.

Days	**Fungi**			
	Beauveria bassiana	***Metarhizium anisopliae***	***Verticillium lecanii***	***Aspergillus flavus***
Day 3	13.38 ± 1.32^D	10.07 ± 1.04^D	7.84 ± 0.76^D	7.29 ± 0.86^D
Day 6	16.86 ± 1.86^C	20.76 ± 2.40^C	17.42 ± 2.25^C	15.20 ± 2.27^C
Day 9	29.17 ± 3.60^B	34.26 ± 3.83^B	29.81 ± 3.53^B	25.92 ± 3.42^B
Day 12	51.62 ± 6.25^A	44.27 ± 5.20^A	40.39 ± 4.66^A	35.94 ± 3.95^A

Similar letters are not significant with each other in a column according to DMRT at $p < 0.05$.

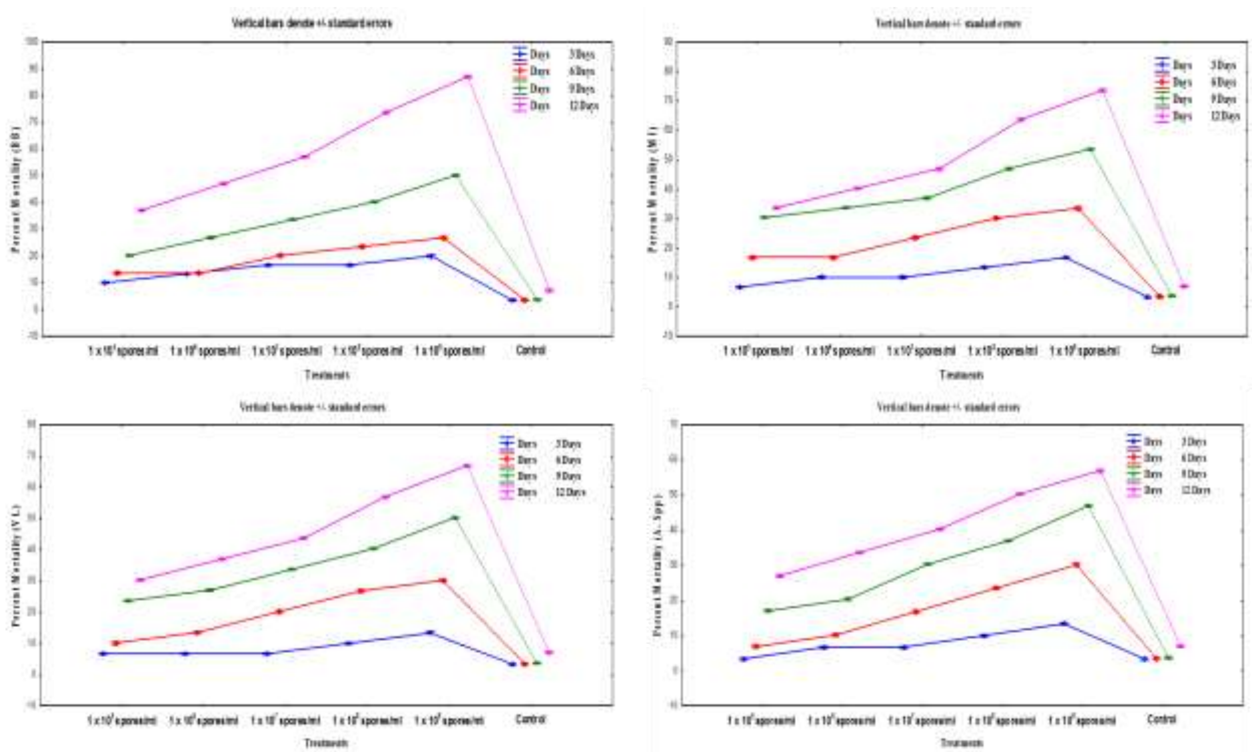

Fig. 1. Interaction b/w different Concentration and Days Interval.

At intervals of 3, 6, 9 and 12 days, all the EPF showed significant effects. At day 12, maximum mortality recorded was 51.62% by *B. bassiana* followed by *M. anisopliae*, *V. lecanii* and *Aspergillus* (44.27%, 40.39%, 35.94%), respectively, while the lowest mortality recorded at day 3 was in range of 13.38%, 10.07%, 7.84%, 7.29%, respectively . There was a direct relation between concentration and days. As concentration increases with days the efficacy of EPFs increased. In case of BB at day 3 with concentration 1×10^5 and 1×10^9 , mortality was 6.8% and 17.2%, respectively while at day 9 with concentration 1×10^5 and 1×10^9 was 32.1 and 85.7, respectively. Similar case was also seen in *M. anisopliae*, *V. lecanii* and *A. flavus*. In *M. anisopliae*, at day 3 with concentration 1×10^5 and 1×10^9, mortality was 3.44 and 13.7% while at day 9 with concentration 1×10^5 and 1×10^9, mortality was 28.5 and 71.4%, respectively. In *V. lecanii*, at day 3 with concentration 1×10^5 and 1×10^9, mortality was 3.44 and 10.3% while at day 9 with concentration 1×10^5 and 1×10^9, mortality was 25 and 64.2% respectively. In case of *A. flavus*, at day 3 with concentration 1×10^5 and 1×10^9, mortality was 3.44 and 10.3% while at day 9 with concentration 1×10^5 and 1×10^9, mortality was 21.4 and 53.5%, respectively.

DISCUSSION

The EPFs with higher concentration showed effective results after treatments within 3, 6, 9 and 12 days. All EPF with high interval rates showed highest efficacy. *B. bassiana*, *V. lecanii*, *M. anisopliae* and *A. flavus* with day interval 12 showed the highest mortality rate of 51.62 ± 6.25. According to Hamadah *et al*. (2018), enzyme activity is believed to be one of the main ways that fungi infect insect hosts and cause desiccation, which results in death. EPFs at varying exposure intervals resulted in notable mortality rates among treated 2nd instar *P. gossypiella* larvae because of their effects on particular hydrolytic enzymes that impact the cuticle, such as lipase, chitinase, and proteinase (Kurtti and Keyhani, 2008; Omar *et al*., 2021). By dissolving the insect cuticle's protein connections and/or utilizing host proteins as food, proteases are hypothesized to help fungal hyphae penetrate host tissue (Pozo *et al*., 2004; Boucias *et al*., 2012). Another reason for the toxic activity of fungal isolates is the total destruction of the fat bodies, which causes the fat bodies to lose their ability to create and store nutrition (Guimaraes and Venancio, 2022; Rousta *et al*., 2024). The results showed that B. bassiana was more effective than the other three EPF. Farooq *et al*. (2020), discovered that *B. bassiana, V. lecanii,* and *M. anisopliae*, both separately and in combination with *Azadirachta indica*, caused significant mortality in treated pink bollworm second instars at varying concentrations and intervals. These outcomes are consistent with the work of others who found that pupation and adult emergence of *C. partellus* 2nd and 4th instar were maximal at low concentrations of entomopathogens (both alone and in combination), whereas they were minimal at high concentrations (Sufyan *et al*., 2019; Rizwan *et al*., 2021; Kebede *et al*., 2022; Sarkar *et al*., 2024). The EPF *B. bassiana, V. lecanii, M. anisopliae,* and *A. flavus* were shown to be active in the study. Therefore, using EPF as an alternative to synthetic pesticides as a control method is a possibility. There is a need for additional field research.

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