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## Research Article

# Evaluation of entomopathogenic fungi for biological control of wheat aphid (*Sitobion avenae*) under laboratory conditions

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## ABSTRACT

A major wheat pest that causes enormous financial losses worldwide is the wheat aphid (*Sitobion avenae*). Aphid populations have traditionally been controlled with chemical pesticides, but because of their negative effects on the environment and human health, sustainable alternatives must be investigated. In the present study, a comparative evaluation of four fungal biocontrol agents namely *Beauveria bassiana*, *Aspergillus flavus*, *Trichoderma viride* and *Metarhizium anisopliae*, demonstrated their efficacy against wheat aphids across varying spore concentrations ( $1 \times 10^4$  to  $1 \times 10^{12}$  cfu/ml) and treatment durations (2–10 days). Aphids mortality was consistently increased with increase in biocontrol's spore concentration and exposure time. For *B. bassiana*, maximum mortality (80%) was observed at  $1 \times 10^{12}$  cfu/ml after 10 days. Similarly, *A. flavus* showed the highest mortality (77%) at the same concentration and time duration. *T. viride* and *M. anisopliae* also caused significant mortality of aphids (60% and 67% respectively) under the same conditions. Lower spore concentrations and time duration exhibited proved less efficient. In conclusion, *B. bassiana* exhibited the highest aphid mortality, highlighting its potential as an effective biocontrol agent.

**Keywords:** *Aspergillus flavus*, *Beauveria bassiana*, biological control, *Metarhizium anisopliae*, *Sitobion avenae*, *Trichoderma viride*.

## INTRODUCTION

Wheat (*Triticum aestivum*) is the most significant agricultural crop in Pakistan, driving the country's economy and means of survival. By employing about 38.5% of the labor force and making up about 19.3% of the GDP, it directly benefits the rural population (Government of Pakistan, 2022). Because it supplies essential raw materials to major industries like food processing, textiles, and sugar that depend on crops like cotton, sugarcane, wheat, rice, and maize, the industry has an impact on more than just rural lifestyles (Pakistan Bureau of Statistics, 2021). About 60% of the population's daily calorie intake comes from the nation's annual production of about 25 million metric tons of wheat (Rafiq et al., 2020). More than 80% of the nation's wheat is produced in the provinces of Punjab and Sindh, which are the main locations for both irrigated and rain-fed wheat cultivation (Ministry of National Food Security and Research, 2021). Worldwide, *Sitobion avenae* (Fab.) (Hemiptera: Aphididae) is a significant insect pest of wheat. It significantly lowers yield by



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attacking at early stages of the wheat crop and feeding on the crop directly, and indirectly spreading wheat diseases (Kindler et al., 1995; Hussain et al., 2024). Some aphid species have developed resistance due to the abuse of pesticides. Thus, it is essential to control their population effectively by using environment friendly alternatives (Shah et al., 2017). Wheat aphids can lower wheat yield by as much as 20-80% (Yahya et al., 2017). Significant financial losses have been caused by aphids' presence, migration, or invasion, especially when comparing Pakistan's average wheat yield with that of its neighbors, Bangladesh and India (Khan et al., 2024). Use of insecticides for management of aphid may cause resistance and environmental health issue. Insecticides isolated from entomopathogens and botanical proved to safe and effective alternate substitute of insecticides against aphid (Masood et al., 2023; Ahmad et al., 2024). Use of these entomopathogens drive toxins as well as their products not only reduce cast of production but also proved to environment friendly (Usha et al., 2014; Zhang et al., 2024).

Control of insect pest with the different control measures like cultural, mechanical and used of resistance varieties are effective part of IPM program for insect pest management. (Kogan, 1998; Angon et al., 2023). Use of IPM techniques for insect pest management may enhance production as well as improved environmental health ( Deguine et al., 2021; Angon et al., 2023; Tiwari et al., 2024). Used of resistance varieties, and EPF for pest management also encourage sustainable pest management techniques (Kogan and Bajwa, 1999; Gao et al., 2024). Insecticides resistance may develop in pest by application to extensively and also biodiversity of beneficial insect harmed (Perry et al., 2013, Rafi et al., 2016; Barathi et al., 2024). New concept of bio rational management of insect pest with environment friendly bio insecticides includes toxins, botanicals and mimics of sex hormones also add in IPM programs (Shapiro and Lacey, 2008; Anjum et al., 2024).

Entomopathogenic fungi (EPF) are among the most important entomopathogens of insect pests. Because *Beauveria bassiana* is vigorous against a variety of insect pests, it is frequently used as an EPF. It is a parasite that affects several arthropod species and produces the white Muscardine disease. It grows organically in the soil (Barbarin et al., 2012; Islam et al., 2021; Qayyum et al., 2024). The fungal species *Metarhizium anisopliae* is another important EPF that causes green muscardine illness in insects. It is extensively used for the biological control of a number of insect pest species (Reddy et al., 2014; Ahmad et al., 2022). Some plants have the ability to kill insects. They are frequently less harmful to the environment and their use stops insect resistance from developing (Isman, 2006; Şengül-Demirak and Canpolat, 2022). They can be used as direct sprays, grain protectants, intercropping with the main crop, synergists, and soil supplements for plant parts, according to Prakash et al., (2008). Botanicals are considered especially helpful because so many products are both safe and reasonably priced. Botanicals may be a tempting option to manmade chemical pesticides because they pose less risk to human health. The compatibility of EPF and pesticides may facilitate the selection of appropriate products for IPM programs (Neves et al., 2001; Wang et al., 2024). Such combination treatments can improve control effectiveness by reducing applied quantities, reducing environmental degradation risks, and decreasing the development of pest resistance (Usha et al., 2014; Zhou et al., 2024).

Important elements of integrated pest management for arthropod pests include biological control, cultural strategies, and host plant resilience. Plant material free of pests is ensured by cultural activities such as intercropping, employing varietal combinations, and treating stem cuttings (Tiwari et al., 2024; Wyckhuys et al., 2024). Although host plant resistance is economical and environmentally beneficial, it can be difficult to apply to many crops. Biopesticides, which are usually natural and can be made, are less likely to cause resistance since they leave very little residue and have little adverse effects on mammals (Khursheed et al., 2022; Hezakiel et al., 2024).

Application of entomopathogenic fungi always depend on host susceptibility and mechanism of isolation from different sources. *B. bassiana* and *M. anisopliae* proved to be effective biopesticides can manage long rang insect pest (Gnat et al., 2021; Sharma et al., 2024). Different aphid species were successfully control with *B. bassiana* in different environmental conditions (Javed et al., 2024; Ma et al., 2024). The use of EPF is one biological control technique that may be included into IPM activities. They are an environmentally friendly substitute for chemical pesticides since they don't affect non-target animals (Roy et al., 2006; Hong et al., 2024). Using EPF to manage wheat aphids may reduce the use for chemical pesticides, resulting in more environmentally friendly methods of wheat cultivation. The objective of the current study was to identify the entomopathogenic fungal strains that were most effective in controlling wheat aphids using laboratory bioassays.

## MATERIALS AND METHODS

Present study was carried in IPM laboratory, Department of Entomology, Faculty of Agricultural Sciences, University of the Punjab during 2023-2024. Efficacy of EPF strains viz., fungi *B. bassiana*, *M. anisopliae*, *A. flavus*, and *T. viride*

was checked against *Sitobion avenae* under controlled environmental condition. The fungal strains (*A. flavus*, *T. viride*, *B. bassiana* and *M. anisopliae*) were obtained from the Department of Entomology, University of the Punjab. These entomopathogenic fungi were inoculated and incubated at 28°C for approximately two weeks to achieve fungal growth. To facilitate large-scale cultivation, 39 g of potato dextrose agar was added to 1.0 L of distilled water, and the mixture was thoroughly stirred before autoclaving for 2 hours. Following autoclaving, the medium was allowed to cool to room temperature and poured into sterilized Petri dishes under laminar flow to prevent contamination. To inhibit bacterial growth, two antibiotic capsules were added to each flask of media. The media were allowed to solidify overnight in the laminar flow cabinet. After two weeks of fungal growth, the conidial/spore layer was carefully scraped using a sterile knife and suspended in a Tween solution. The spore concentration was determined using a hemocytometer, and five distinct fungal concentrations were prepared:  $1 \times 10^4$ ,  $1 \times 10^6$ ,  $1 \times 10^8$ ,  $1 \times 10^{10}$ , and  $1 \times 10^{12}$  cfu/ml, using distilled water. The experiment was performed using a completely randomized design and the analysis of variance (ANOVA) was conducted using Statistix 8.1 software. Pairwise comparisons were made using Tukey's HSD test at a 5% significance level to assess the relative efficacy of the treatments. The effectiveness of the fungal strains was determined by comparing the aphid mortality across different fungal concentrations.

## RESULTS

The efficacy of four fungal species was evaluated against wheat aphids at varying spore concentrations ( $1 \times 10^{12}$ ,  $1 \times 10^{10}$ ,  $1 \times 10^8$ ,  $1 \times 10^6$ , and  $1 \times 10^4$  cfu/ml) over a 10-day period. Mortality of aphids was recorded at 2, 4, 6, 8, and 10 days post-treatment. The results showed a consistent increase in aphid mortality with increasing fungal spore concentration and treatment duration for all the four fungal species. For *B. bassiana*, the highest concentration of  $1 \times 10^{12}$  cfu/ml resulted in the highest mortality, starting at 33% after 2 days and increasing to 80% after 10 days. The second-highest concentration ( $1 \times 10^{10}$  cfu/ml) resulted in 27% mortality after 2 days, with a peak of 70% after day 10. Mortality due to lower concentrations viz.  $1 \times 10^8$ ,  $1 \times 10^6$ , and  $1 \times 10^4$  cfu/ml was comparatively lower. The control treatment showed the least mortality at all-time points, with a maximum of  $6.68 \pm 0.22\%$  by day 10. This trend was consistent across all time intervals, indicating a clear dose-response relationship (Table 1).

Table 1. Percentage mortality of wheat aphid due to *B. bassiana*.

Concentration (cfu/ml)	Percentage mortality $\pm$ S.E.				
	2 DAT	4 DAT	6 DAT	8 DAT	10 DAT
$1 \times 10^4$	10 $\pm$ 0.23 <sup>E</sup>	13 $\pm$ 0.25 <sup>E</sup>	23 $\pm$ 0.25 <sup>E</sup>	27 $\pm$ 0.25 <sup>E</sup>	33 $\pm$ 0.25 <sup>E</sup>
$1 \times 10^6$	17 $\pm$ 0.32 <sup>D</sup>	20 $\pm$ 0.33 <sup>D</sup>	27 $\pm$ 0.33 <sup>D</sup>	33 $\pm$ 0.33 <sup>D</sup>	43 $\pm$ 0.33 <sup>D</sup>
$1 \times 10^8$	20 $\pm$ 0.29 <sup>C</sup>	27 $\pm$ 0.28 <sup>C</sup>	37 $\pm$ 0.28 <sup>C</sup>	40 $\pm$ 0.31 <sup>C</sup>	53 $\pm$ 0.31 <sup>C</sup>
$1 \times 10^{10}$	27 $\pm$ 0.45 <sup>B</sup>	37 $\pm$ 0.44 <sup>B</sup>	47 $\pm$ 0.44 <sup>B</sup>	50 $\pm$ 0.64 <sup>B</sup>	70 $\pm$ 0.64 <sup>B</sup>
$1 \times 10^{12}$	33 $\pm$ 0.49 <sup>A</sup>	43 $\pm$ 0.46 <sup>A</sup>	57 $\pm$ 0.55 <sup>A</sup>	60 $\pm$ 0.71 <sup>A</sup>	80 $\pm$ 0.71 <sup>A</sup>
Control	3 $\pm$ 0.21 <sup>F</sup>	3 $\pm$ 0.24 <sup>F</sup>	3 $\pm$ 0.21 <sup>F</sup>	7 $\pm$ 0.21 <sup>F</sup>	7 $\pm$ 0.22 <sup>F</sup>

*Aspergillus flavus* exhibited similar trends in aphid mortality. At the highest concentration ( $1 \times 10^{12}$  cfu/mL), mortality was 27% after 2 days and reached to 77% after 10 days. At  $1 \times 10^{10}$  cfu/ml, mortality was 20% after 2 days, peaking at 67% by day 10. Concentrations of  $1 \times 10^8$ ,  $1 \times 10^6$ , and  $1 \times 10^4$  cfu/ml showed progressively lower mortality percentages, while the control group consistently showing the least mortality. This fungal species displayed a strong concentration-dependent mortality response that was enhanced over time (Table 2).

Table 2. Percentage mortality of wheat aphid due to *T. flavus*.

Concentration (cfu/ml)	Percentage mortality $\pm$ S.E.				
	2 DAT	4 DAT	6 DAT	8 DAT	10 DAT
$1 \times 10^4$	7 $\pm$ 0.25 <sup>E</sup>	10 $\pm$ 0.25 <sup>E</sup>	17 $\pm$ 0.27 <sup>E</sup>	20 $\pm$ 0.22 <sup>E</sup>	27 $\pm$ 0.28 <sup>E</sup>
$1 \times 10^6$	137 $\pm$ 0.33 <sup>D</sup>	17 $\pm$ 0.33 <sup>D</sup>	23 $\pm$ 0.33 <sup>D</sup>	27 $\pm$ 0.34 <sup>D</sup>	40 $\pm$ 0.33 <sup>D</sup>
$1 \times 10^8$	17 $\pm$ 0.39 <sup>C</sup>	23 $\pm$ 0.37 <sup>C</sup>	33 $\pm$ 0.41 <sup>C</sup>	37 $\pm$ 0.37 <sup>C</sup>	50 $\pm$ 0.59 <sup>C</sup>
$1 \times 10^{10}$	20 $\pm$ 0.44 <sup>B</sup>	33 $\pm$ 0.47 <sup>B</sup>	37 $\pm$ 0.47 <sup>B</sup>	50 $\pm$ 0.50 <sup>B</sup>	67 $\pm$ 0.68 <sup>B</sup>
$1 \times 10^{12}$	27 $\pm$ 0.29 <sup>A</sup>	37 $\pm$ 0.49 <sup>A</sup>	43 $\pm$ 0.49 <sup>A</sup>	60 $\pm$ 0.61 <sup>A</sup>	77 $\pm$ 0.81 <sup>A</sup>
Control	3 $\pm$ 0.22 <sup>F</sup>	3 $\pm$ 0.22 <sup>F</sup>	3 $\pm$ 0.23 <sup>F</sup>	7 $\pm$ 0.50 <sup>F</sup>	7 $\pm$ 0.25 <sup>F</sup>

For *T. viride*, aphid mortality at  $1 \times 10^{12}$  cfu/ml was 23% after 2 days and increased to 60 % by day 10. Lower concentrations of  $1 \times 10^{10}$ ,  $1 \times 10^8$ ,  $1 \times 10^6$ , and  $1 \times 10^4$  cfu/ml showed progressively lower mortality. The highest concentration showed a significant increase in efficacy over the 10-day period, similar to the trends observed in *B. bassiana* and *A. flavus* (Table 3).

Table 3. Percentage mortality of wheat aphid (*Sitobin avenae*) due to *T. viride*.

Concentration (cfu/ml)	Percentage mortality $\pm$ S.E.				
	2 DAT	4 DAT	6 DAT	8 DAT	10 DAT
$1 \times 10^4$	7 $\pm$ 0.25 <sup>E</sup>	10 $\pm$ 0.22 <sup>E</sup>	17 $\pm$ 0.29 <sup>D</sup>	23 $\pm$ 0.25 <sup>D</sup>	30 $\pm$ 0.23 <sup>E</sup>
$1 \times 10^6$	10 $\pm$ 0.34 <sup>D</sup>	17 $\pm$ 0.30 <sup>D</sup>	23 $\pm$ 0.30 <sup>C</sup>	30 $\pm$ 0.31 <sup>C</sup>	37 $\pm$ 0.35 <sup>D</sup>
$1 \times 10^8$	13 $\pm$ 0.32 <sup>C</sup>	20 $\pm$ 0.35 <sup>C</sup>	27 $\pm$ 0.38 <sup>B</sup>	37 $\pm$ 0.36 <sup>B</sup>	43 $\pm$ 0.41 <sup>C</sup>
$1 \times 10^{10}$	17 $\pm$ 0.51 <sup>B</sup>	23 $\pm$ 0.44 <sup>B</sup>	27 $\pm$ 0.44 <sup>B</sup>	37 $\pm$ 0.39 <sup>B</sup>	47 $\pm$ 0.49 <sup>B</sup>
$1 \times 10^{12}$	23 $\pm$ 0.31 <sup>A</sup>	37 $\pm$ 0.49 <sup>A</sup>	43 $\pm$ 0.51 <sup>A</sup>	50 $\pm$ 0.49 <sup>A</sup>	60 $\pm$ 0.71 <sup>A</sup>
Control	4 $\pm$ 0.22 <sup>F</sup>	4 $\pm$ 0.23 <sup>F</sup>	4 $\pm$ 0.23 <sup>E</sup>	7 $\pm$ 0.23 <sup>E</sup>	7 $\pm$ 0.22 <sup>F</sup>

Lastly, *M. anisopliae* exhibited the most substantial effect on aphid mortality among the fungal species tested. At the highest concentration ( $1 \times 10^{12}$  cfu/ml), mortality was 30 % after 2 days and reached to 67 % by day 10. Mortality due to  $1 \times 10^{10}$ ,  $1 \times 10^8$ ,  $1 \times 10^6$ , and  $1 \times 10^4$  cfu/ml increased over time but remained lower than those observed for the highest concentration. The control treatment once again showed the lowest mortality at all-time points. This fungal species exhibited a strong concentration-dependent response, with notable increases in efficacy over time (Table 4).

Table 4. Percentage mortality of wheat aphid (*Sitobin avenae*) due to *M. anisopliae*.

Concentration (cfu/ml)	Percentage mortality $\pm$ S.E.				
	2 DAT	4 DAT	6 DAT	8 DAT	10 DAT
$1 \times 10^4$	10 $\pm$ 0.23 <sup>E</sup>	17 $\pm$ 0.23 <sup>E</sup>	20 $\pm$ 0.23 <sup>E</sup>	27 $\pm$ 0.32 <sup>E</sup>	33 $\pm$ 0.23 <sup>E</sup>
$1 \times 10^6$	14 $\pm$ 0.32 <sup>D</sup>	20 $\pm$ 0.32 <sup>D</sup>	23 $\pm$ 0.32 <sup>D</sup>	30 $\pm$ 0.32 <sup>D</sup>	37 $\pm$ 0.32 <sup>D</sup>
$1 \times 10^8$	17 $\pm$ 0.29 <sup>C</sup>	23 $\pm$ 0.29 <sup>C</sup>	33 $\pm$ 0.29 <sup>C</sup>	40 $\pm$ 0.29 <sup>C</sup>	50 $\pm$ 0.29 <sup>C</sup>
$1 \times 10^{10}$	27 $\pm$ 0.45 <sup>B</sup>	37 $\pm$ 0.45 <sup>B</sup>	47 $\pm$ 0.45 <sup>B</sup>	50 $\pm$ 0.45 <sup>B</sup>	57 $\pm$ 0.45 <sup>B</sup>
$1 \times 10^{12}$	30 $\pm$ 0.49 <sup>A</sup>	40 $\pm$ 0.49 <sup>A</sup>	53 $\pm$ 0.49 <sup>A</sup>	67 $\pm$ 0.49 <sup>A</sup>	67 $\pm$ 0.49 <sup>A</sup>
Control	4 $\pm$ 0.21 <sup>F</sup>	4 $\pm$ 0.21 <sup>F</sup>	4 $\pm$ 0.21 <sup>F</sup>	7 $\pm$ 0.21 <sup>F</sup>	7 $\pm$ 0.21 <sup>F</sup>

## DISCUSSION

Because of worries about how chemical pesticides affect the environment, entomopathogenic fungi have drawn a lot of interest as effective biological control agents for aphids, especially *Sitobion avenae* (wheat aphid). The purpose of this study was to evaluate the efficacy of four entomopathogenic fungi namely *T. viride*, *A. flavus*, *B. bassiana* and *M. anisopliae*, against wheat aphids in a lab setting. With a mortality of up to 80%, *B. bassiana* was found to be the most effective, followed by *A. flavus* (76%), *T. viride* (60%) and *M. anisopliae* (66%). The best performance of *B. bassiana* in this investigation is consistent with the results of earlier studies. Aphids are among the many insect pests that *B. bassiana* is known to be broad-spectrum pathogenic against. Shah and Pell (2003) demonstrated the effectiveness of *B. bassiana* against aphids. Present finding were in line with Feng et al. (2021), in his study report they demonstrated that *B. bassiana* was effectively control peach aphids and mortality range was 75-80%. This mortality was achieved different enzymes and their productions which was isolated form dead cadavers reported by (Inglis et al., 2001).

Entomopathogenic fungi *A. flavus* caused 70-80% mortality on wheat aphid and categorized as 2<sup>nd</sup> highest biopesticides for causing highest mortality. Previous studies also conform similar result as describe Zarrin et al. (2019) who's reported that cotton aphid was controlled effectively by application of *A. flavus* and mortality range was 72-75%. *A. flavus* was also effect for plant health and controlling different plant pathogens. Report given by Harman et al. (2004) and Akram et al. (2020), describe that *A. flavus* mortality of *Sitophilus oryzae* was maximum 76-80% which was comparable with mortality caused by *B. bassiana*.

Maximum mortality of aphid was observed on treatment treated with *M. anisopliae*. Mortality range was 60-70% which was also recorded by Goettel et al. (1990) whose reported that wheat aphid was effectively controlled up to 60% by application of *M. anisopliae* under controlled environmental condition. Similar results were also reported by Ullah et al. (2021) whose reported that 70% mortality of which aphid was recorded after application *M. anisopliae*.

Results revealed that, application of *B. bassiana*, *M. anisopliae*, *A. flavus*, and *T. viride* was most effective for managing which aphid under controlled environmental conditions. These results proved that EPF strains viz., *B. bassiana*, *M. anisopliae*, *A. flavus*, and *T. viride* was successfully incorporated in IPM program and also other field experiments.

## CONCLUSIONS

Four EPF stains, *B. bassiana*, *M. anisopliae*, *A. flavus*, and *T. viride* was most effective for management of wheat aphid. Study revealed that, at height concentration ( $1 \times 10^{12}$  cfu/ml) *B. bassiana* cause 80-85%, followed by 75-80%, 65-70% and 60-65% mortality caused by *A. flavus*, *M. anisopliae* and *T. viride* respectively. Hence EPF strains was most economical and environment friendly pest management tactic and recommended as effective part of IPM program.

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## AUTHOR CONTRIBUTIONS

Arfa Yaqoob, Mubashar Iqbal and Shahbaz Ahmad: Created research plan, analyzed the data and wrote the manuscript. Arshad Javaid, Muhammad Ashfaq, Muhammad Bilal Chattha and Sumreen Anjum: Critically analyzed the data and played advisory role in research plan and statistical analysis.

## COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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