Research Article

Efficacy of *Beauveria bassiana* and *Metarhizium anisopliae* for the Management of Tomato Fruit Borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract |The present experiment was conducted to evaluate the effect of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Hypocreales) against tomato fruit borer, *Helicoverpa armigera* under controlled laboratory conditions. Three different concentrations viz., 1×10^8, 5×10^7 and 1×10^7 spores/ml of tested entomopathogenic fungi i.e., *B. bassiana* and *M. anisopliae* were prepared in the laboratory and inoculated on the dorsal side of 3rd instar larvae of *H. armigera* by using a micro-pipette. In other experiment, tomato nursery was planted in pots and prepared concentrations (1×10^8, 5×10^7 and 1×10^7 spores/ml) of above mentioned entomopathogenic fungi were sprayed on tomato plants (5-7 leave stage) including control (water) by hand pump sprayer. The treated plants were shifted in insect cages and two pairs of *H. armigera* adults were released in each cage for egg laying. Results showed that earliest mean mortality and total corrected mortality of *H. armigera* i.e., after 3.33 days and 86.86%, respectively was recorded for *B. bassiana* @ 1×10^8 spores/ml. Probit analysis showed that the LC_{50} value recorded for *B. bassiana* and *M. anisopliae* was 1.721×10^7 and 3.966×10^7 spores/ml, respectively, while, LT_{50} value recorded for *B. bassiana* and *M. anisopliae* was 10.230 and 10.496 days, respectively. The maximum fecundity and egg hatching (%) was recorded in control treatment i.e., 103.33 eggs and 86.30%, respectively, while, minimum egg laying and egg hatching was recorded in *B. bassiana* @ 1×10^8 spores/ml i.e., 21.00 eggs and 49.58%, respectively. It is concluded that the use of *B. bassiana* and *M. anisopliae* at a concentration of 1×10^8 spores/ml offers a promising and eco-friendly solution for controlling *H. armigera* in vegetable crops.

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Introduction

Tomato is one of the most valuable short-term vegetable crops in the world. Tomatoes are a nutrient-dense food that contains vitamins A, B, and C, minerals, amino acids, sugars, and dietary fiber (Khokhar and HRI, 2013). In 2022, the global tomato planting area was 05 million hectares, with a total

output of 186.82 million tons (FAOSTAT, 2022). China is the main producer of tomatoes accounting for 34.72% of the total global production followed by India, Turkey, United States, and Egypt. Pakistan has been ranked as the 26th largest tomato producer in the world (Anonymus, 2022; Tiwari *et al*., 2022).

In Pakistan, tomatoes are grown in all provinces in spring and autumn, but unfortunately the net productivity is very low compared to the rest of the world. The tomato is a tropical plant that is well adapted to almost all climate zones in the world; however, environmental stress significantly reduces yield potential due to environmental stress. Abiotic stresses including high temperature, chilling, excessive light, toxic ions, water logging, wounding, osmotic shock, drought, salinity, exposure to ozone and radiation (UV-B) and biotic stresses including arthropod pests, fungal, bacterial and viral diseases are responsible for low yield in tomato crops (Kubienova *et al*., 2013).

Among arthropod pests, the most important pests infesting tomato crop are the fruit borer (*Helicoverpa armigera*), leafminer moth (*Tuta absoluta Meyrick*), whiteflies (*Bemisia tabaci Gennadius*), thrips (*Frankiliniella occidentalis Pergade*), aphids (*Myzus persicae*) and mites (Tetranychus spp) (Wakil *et al*., 2018). The fruit borer, *H. armigera* (Hübner) is a destructive pest of tomato plants severely hinders crop yield and productivity, damages developing fruit and causes 20 to 60% fruit loss (Talekar *et al*., 2006; Saljoqi *et al*., 2022).

H. armigera is a globally important pest affecting many crops, causing severe economic losses to pigeon pea, soybean, sorghum, maize, tomato, cotton, flaxseed, pea, chickpea and many vegetables (Ali *et al*., 2020). As it is polyphagous in nature and due to lack of environmental friendly different managing strategies, many farmers around the world especially in Pakistan rely on synthetic pesticides to cope with this destructive pest (Dagne *et al*., 2018). Beta-cyhalothrin and dimethoate are commonly used insecticides for the control of *H. armigera* in many countries (Fite *et al*., 2024). However, excessive use of pesticides may cause severe environmental pollution, can pose very harmful effects on beneficial organisms and human beings, increased production costs and ecological imbalances (Macharia, 2015; Khalique *et al*., 2018). In addition, *H. armigera* become resistant to nearly all commonly used conventional insecticides (Sun *et al*., 2019).

Pesticide selection pressure can be reduced by substituting synthetic pesticides with biopesticides in IPM strategies pointing this pest (Majeed *et al*., 2018). The use of EPFs (entomopathogenic fungi) as insect biocontrol agents has received worldwide attention (Majeed *et al*., 2018). Numerous studies have evaluated EPFs-biomolecules in integrated pest management strategies as an alternative to synthetic pesticides (Batool *et al*., 2022).

Biopesticides from *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycota: Hypocreales) have been used to reduce important agricultural insect pests worldwide (Ahmad *et al*., 2019; Batool *et al*., 2022). *M. anisopliae* and *B. bassiana* were characterized for nematodes (Reinbacher *et al*., 2021), tomato fruit worm (Kary *et al*., 2022), *Agrotis ipsilon* (El-Hawary, 2019), and several other pests (Ahmed *et al*., 2023). In view of the current challenges in managing *H. armigera* in tomato crops, the use of entomopathogenic fungi, namely *M. anisopliae and B. bassiana* presents a best control. The objectives of the current research were focused on: i) to evaluate the efficacy of *M. anisopliae* and *B. bassiana* against *H. armigera* larvae under controlled conditions. ii) to determine the effect of *M. anisopliae* and *B. bassiana* on ovipositional preference of *H. armigera* on tomato plants.

Materials And Methods

The current research was conducted in bio-control laboratory, Plant Protection Division at Nuclear Institute for Food and Agriculture (NIFA), Peshawar during 2021-22. Laboratory conditions were maintained at 25±2°C temperature and 65±5% R.H. with 08:16 hrs. (L:D) photoperiod.

Insect culture

An initial stock of fruit worm larvae were collected from the chickpea fields (34.0155° N and 71.7129° E) during the months of December-February, 2021-22 from the field area of Nuclear Institute for Food and Agriculture and reared on natural diet in controlled laboratory conditions (Temp. 25±2°C; 65±5% R.H.; 08:16 h. (L:D) photoperiod) for the culture maintenance before conducting the experiment. After adult emergence, these were paired to get eggs

and neonate larvae. Counted number of 3rd instar larvae or newly emerged adults (un-sexed) were used/released in the experiments.

Plant material

Tomato plants (Lariqa cultivar) were selected for the experiment. For this, tomato nursery was obtained from Tarnab farm nursery area, Peshawar and kept under controlled conditions. Nursery was covered with transparent plastic bags to prevent any interruption. Plants with 5-7 leave stage were used for the experiment.

Fungus culture

M. anisopliae (PACER®) and *B. bassiana* (RACER®) manufactured by AgriLife SOM Phytopharma (India) Limited (www.agrilife.in) were obtained from University of Agriculture, Faisalabad and evaluated against fruit worm larvae and adults. Different concentrations (1×10^8, 5×10^7 and 1×10^7 spores/ml) of *M. anisopliae* and *B. bassiana* were prepared in distilled water and were used against 3rd instar larvae of *H. armigera* and ovipositional preference of their adults.

Experimental layout

Evaluation of EPF's against tomato fruit worm larva

Laboratory experiment was carried out during April-June, 2022 to determine the efficacy of *M. anisopliae*, and *B. bassiana* against fruit worm larvae. Different concentrations of both entomopathogenic fungi were formed in distilled water and water alone was considered as a control treatment (Table 1).

Table 1: *List of treatments and their concentrations used in the experiment.*

Treatment	Active ingredient	Concentration (No. of Spores)
T1	*Metarhizium anisopliae*	1×10^8
T2	*Metarhizium anisopliae*	5×10^7
T3	*Metarhizium anisopliae*	1×10^7
T4	*Beauveria bassiana*	1×10^8
T5	*Beauveria bassiana*	5×10^7
T6	*Beauveria bassiana*	1×10^7
T7	Water	

Each treatment were applied on five 3rd instar larvae of tomato fruit worm by topically inoculation with fresh conidial suspensions. Each larva was topically inoculated with 10 µl of conidial suspensions by using a micropipette (Eppendorf pipettes, 1-20 µl). In control treatment, larvae were treated with distilled water only. Soon after treatment application, larvae were fed with fresh natural diet which was changed daily. Completely randomized design (CRD) was followed with three replications of each treatment.

First mortality

After the application of tested EPFs (*B. bassiana* and *M. anisopliae*), the mortality data was recorded for ten days. The first mortality of tomato fruit worm larva was recorded in replications of each treatment. Larva was considered dead when it was disturbed with camel hairbrush and no sign of movement was observed.

Total corrected mortality

Mortality data of tomato fruit worm larva was recorded in replications of each treatment and corrected mortality data was designed by using Abbott formula (Abbott, 1925) as follows:

$$\text{Corrected \% mortality} = \left(1 - \frac{\text{n in T after treatment}}{n \text{ in Co after treatment}}\right) \times 100$$

Where: n = Insect population, T = Treated, Co = Control

Fungal growth

For confirmation, dead larvae were placed in sterile sealed Petri dishes with moist filter paper to promote fungal growth. As a result of the experimental treatment, signs of mycelial growth and conidia formation on the cadavers were observed.

Pupal recovery and pupal weight

Surviving larvae were kept in petri dishes to form pupae. Sex differentiation was determined at the pupal stage based on the presence or absence of small black dots on top of the abdomen of males and females, and pupal weights were also recorded. The percentage of pupae recovery was calculated using the following formula:

$$\text{Pupae recovery (\%)} = \frac{\text{Number of pupae formed}}{\text{Total number of larvae}} \times 100$$

Adult emergence

After pupation, pupae were placed in separate clean Petri plates using forceps and were kept in plastic

containers for eclosion. Percent adult emergence (eclosion) was calculated as:

$$\text{Adult emergence (\%)} = \frac{\text{Number of emerged adults}}{\text{Total number of pupae}} \times 100$$

Lethal concentration and lethal time to calculate 50% mortality of fruit worm larva

All larval mortality counts were analysed by Probit using SPSS v16 software. The efficacy of tested entomopathogenic fungi was compared on the basis of median lethal concentration (LC_{50}) and lethal time (LT_{50}).

Regression analysis between mean mortality and tested M. anisopliae and B. bassiana

Data obtained on mortality percentage of tomato fruit worm larvae by the two fungi (*M. anisopliae* and *B. bassiana*) were used for plotting a linear relationship between the mortality percentage and the days after treatments, using different concentrations of tested fungi. Regression equations were calculated by conducting regression analysis.

Evaluation of entomopathogenic fungi against ovipositional preference

Free choice bioassay was conducted to assess the ovipositional preference of fruit worm moths on tomato plants with 5-7 leave stage. Plants were sprayed with given concentrations of *M. anisopliae* and *B. bassiana* as shown in Table 1 using suitable hand pump sprayer. Plant treated with water alone was considered as controlled treatment. Counted number of fruit worm adults were released in each replication to assess the ovipositional preference. In this bioassay, treated and untreated plants were kept in same cage. Completely randomized design was followed with three replications of each treatment. After three days, plants with eggs were removed from the cage and each plant was kept in separate cage for the data recording of following parameters:

Egg hatch rate (%)

Data regarding egg laying numbers of tomato fruit worm adults on treated and untreated plants were recorded on daily basis till the death of the adults. Egg hatch rate (%) was calculated by using the below mentioned formula:

$$\text{Egg hatch rate (\%)} = \frac{\text{No of hatched eggs}}{\text{Total No of eggs}} \times 100$$

Tomato yield

On maturity of tomato plants, data regarding number of total fruits and damaged fruits per plant were recorded for percent damage assessment of each treated and untreated tomato plants by using the following formula:

$$\text{Percent Damage Assessment} = \frac{\text{Number of damaged tomato fruits}}{\text{Total number of tomato fruits}} \times 100$$

Data regarding percent yield increase over control treatment was calculated by following formula:

$$\text{Percent Yield Increase} = \frac{\text{Weight of fresh tomato in } T_t - \text{Weight of fresh tomato in } T_0}{\text{Weight of fresh tomato in } T_0} \times 100$$

Where, Tt = Treated and T0 = Control.

Statistical analysis

Recorded data were analyzed by using statistical software "Statistix 8.1" through ANOVA and mean were compared by using Least Significance Difference (LSD) test at 5% level of significance. Probit analysis was performed to calculate LC_{50} and LT_{50} of tested entomopathogenic fungi by using statistical software "SPSS v16".

Results

First mortality

M. anisopliae and *B. bassiana* were evaluated against the first mortality of tomato fruit worm, *Helicoverpa armigera* in laboratory. Experimental results showed that the treatments caused significant mortality of *H. armigera* larvae under controlled conditions ($P < 0.05$; $F = 17.7$). Comparison of each treatment with mean values (days) and standard errors showed that the earliest mean mortality of *H. armigera* i.e., 3.33 days was recorded in *B. bassiana* @ 1×10^8 spores/ml followed by *B. bassiana* @ 5×10^7 spores/ml (5.00 days), *M. anisopliae* @ 1×10^8 spores/ml (7.33 days), *M. anisopliae* @ 5×10^7 spores/ml (9.33 days), *B. bassiana* @ 1×10^7 spores/ml (10.67 days) and *M. anisopliae* @ 1×107 spores/ml (12.67 days) while late mortality was recorded in control treatment i.e., 15.67 days (Table 2).

Table 2: *Mean table for first mortality (days) of H. armigera in different entomopathogenic treatments.*

Sr. No.	Treatment	First mortality (days) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	7.33 ± 0.40 de
T2	*M. anisopliae* (5 × 10^7 spores/ml)	9.33 ± 0.67 cd
T3	*M. anisopliae* (1 × 10^7 spores/ml)	12.67 ± 0.88 ab
T4	*B. bassiana* (1 x 10^8 spores/ml)	3.33 ± 0.99 f
T5	*B. bassiana* (5 × 10^7 spores/ml)	5.00 ± 1.73 ef
T6	*B. bassiana* (1 × 10^7 spores/ml)	10.67 ± 0.77 bc
T7	Control (water)	15.67 ± 1.20 a
	LSD value	3.10

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Corrected mortality

M. anisopliae and *B. bassiana* were evaluated against the maximum corrected mortality of tomato fruit worm, *H. armigera* in laboratory. Experimental results showed that the treatments caused significant mortality of *H. armigera* larvae under controlled conditions (P < 0.05; F = 7.80). Comparison of each treatment with mean values (% mortality) and standard errors showed that the minimum corrected mortality of *H. armigera* i.e., 26.80% was recorded in *M. anisopliae* @ 1×10^7 spores/ml followed by *B. bassiana* @ 1×10^7 spores/ml (40.07%), *M. anisopliae* @ 5×10^7 spores/ml (46.80%), *B. bassiana* @ 5×10^7 spores/ml (66.93%) and *M. anisopliae* @ 1×10^8 spores/ml (73.53%) while maximum mean corrected mortality was recorded in *B. bassiana* @ 1×10^8 spores/ml i.e., 86.86% (Table 3).

Table 3: *Mean table for corrected mortality (%) of H. armigera in different entomopathogenic treatments.*

Sr. No.	Treatment	Corrected mortality (%) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	73.53 ± 8.67 a
T2	*M. anisopliae* (5 × 10^7 spores/ml)	46.80 ± 6.60 bc
T3	*M. anisopliae* (1 × 10^7 spores/ml)	26.80 ± 6.80 c
T4	*B. bassiana* (1 x 10^8 spores/ml)	86.86 ± 8.63 a
T5	*B. bassiana* (5 × 10^7 spores/ml)	66.93 ± 9.02 ab
T6	*B. bassiana* (1 × 10^7 spores/ml)	40.07 ± 8.60 c
	LSD value	24.99

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Pupal recovery

M. anisopliae and *B. bassiana* were evaluated against the pupae recovery (%) of tomato fruit worm, *H. armigera* from alive larvae in laboratory. Experimental results showed that the treatments have significant pupae recovery *of H. armigera* under controlled conditions (P < 0.05; F = 5.37). Comparison of each treatment with mean values and standard errors showed that the minimum pupae recovery (%) of *H. armigera* i.e., 6.67% was recorded in *B. bassiana* @ 1×10^8 spores/ml followed by *M. anisopliae* @ 1×10^8 spores/ml (20.00%), *B. bassiana* @ 5×10^7 spores/ml (26.67%), *M. anisopliae* @ 5×10^7 spores/ml (40.00%), *B. bassiana* @ 1×10^7 spores/ml (53.33%) and *M. anisopliae* @ 1×10^7 spores/ml (66.67%) while maximum pupae recovery was recorded in control treatment (water) i.e., 93.33% (Table 4).

Table 4: *Mean table for pupae recovery (%) of H. armigera in different entomopathogenic treatments.*

Sr. No.	Treatment	Pupae recovery (%) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	20.00 ± 11.55 cd
T2	*M. anisopliae* (5 × 10^7 spores/ml)	40.00 ± 11.55 bcd
T3	*M. anisopliae* (1 × 10^7 spores/ml)	66.67 ± 13.33 ab
T4	*B. bassiana* (1 x 10^8 spores/ml)	6.67 ± 6.67 d
T5	*B. bassiana* (5 × 10^7 spores/ml)	26.67 ± 17.63 cd
T6	*B. bassiana* (1 × 10^7 spores/ml)	53.33 ± 17.63 bc
T7	Control (water)	93.33 ± 6.67 a
	LSD value	38.97

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Pupal weight

M. anisopliae and *B. bassiana* were evaluated against the pupae weight (gm) of tomato fruit worm, *H. armigera* in laboratory. Experimental results showed that the treatments have significant pupae weight of *H. armigera* under controlled conditions (P < 0.05; F = 18.5). Comparison of each treatment with mean values and standard errors showed that the minimum pupae weight (gm) of *H. armigera* i.e., 0.19 gm was recorded in *B. bassiana* @ 1×10^8 spores/ml followed by *B. bassiana* @ 5×10^7 spores/ml (0.24 gm), *B. bassiana* @ 1×10^7 spores/ml (0.25 gm), *M. anisopliae* @ 1×10^8 spores/ml (0.27 gm), *M. anisopliae* @ 5×10^7 spores/ml (0.27 gm) and *M. anisopliae* @ 1×10^7 spores/ml (0.30 gm) while maximum pupae weight was recorded in control treatment (water) i.e., 0.32 gm (Table 5).

Adult emergence

M. anisopliae and *B. bassiana* were evaluated against the adult emergence of tomato fruit worm, *H.*

armigera in laboratory.

Table 5: *Mean table for pupae weight (gm) of H. armigera in different entomopathogenic treatments.*

Sr. No.	Treatment	Pupae weight (mg) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	0.27 ± 0.01 b
T2	*M. anisopliae* (5 × 10^7 spores/ml)	0.27 ± 0.02 b
T3	*M. anisopliae* (1 × 10^7 spores/ml)	0.30 ± 0.00 a
T4	*B. bassiana* (1 x 10^8 spores/ml)	0.19 ± 0.00 d
T5	*B. bassiana* (5 × 10^7 spores/ml)	0.24 ± 0.01 c
T6	*B. bassiana* (1 × 10^7 spores/ml)	0.25 ± 0.00 bc
T7	Control (water)	0.32 ± 0.01 a
	LSD value	0.03

Means within columns followed by the different lowercase letters are significantly different at P < 0.05

Experimental results showed the significant adult emergence of *H. armigera* under controlled conditions (P < 0.05; F = 5.92). Comparison of each treatment with mean values and standard errors showed that the minimum or no adult emergence (%) of *H. armigera* i.e., 0.00 % was recorded in *B. bassiana* @ 1×10^8 spores/ml followed by *B. bassiana* @ 5×10^7 spores/ml (11.11%), *B. bassiana* @ 1×10^7 spores/ml (38.89%), *M. anisopliae* @ 1×10^8 spores/ml (50.00%), *M. anisopliae* @ 5×10^7 spores/ml (83.33%) and *M. anisopliae* @ 1×10^7 spores/ml (86.67%) while maximum mean adult emergence was recorded in control treatment (water) i.e., 88.89% (Table 6).

Table 6: *Mean table for adult emergence of H. armigera in different entomopathogenic treatments.*

Sr. No.	Treatment	Adult emergence ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	50.00 ± 28.87 abc
T2	*M. anisopliae* (5 × 10^7 spores/ml)	83.33 ± 8.33 ab
T3	*M. anisopliae* (1 × 10^7 spores/ml)	86.67 ± 6.67 a
T4	*B. bassiana* (1 x 10^8 spores/ml)	0.00 ± 0.00 d
T5	*B. bassiana* (5 × 10^7 spores/ml)	11.11 ± 11.11 cd
T6	*B. bassiana* (1 × 10^7 spores/ml)	38.89 ± 20.03 bcd
T7	Control (water)	88.89 ± 11.11 a
	LSD value	45.79

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Regression analysis between mean mortality and tested entomopathogenic fungi

Linear regression analysis was conducted between mean mortality of tomato fruit worm and different concentrations of *M. anisopliae*. The data indicates that the calculated regression equations and R2 values of the tested entomo-pathogenic i.e., *M. anisopliae* as follows: for T1, y = 4.5057x, R2 = 0.5938; T2, y = 3.3498x, R2 = 0.8677; T3, y = 1.6749x, R2 = 0.5867 at intercept = 0 (Figure 1). Linear regression analysis was also conducted between mean mortality of tomato fruit worm and different concentrations of *B. bassiana*. The data indicates that the calculated regression equations and R2 values of the tested entomo-pathogenic i.e., *B. bassiana* as follows: for T1, y = 4.9787x, R2 = 0.7621; T2, y = 3.422x, R2 = 0.7226; T3, y = 1.931x, R2 = 0.613 at intercept = 0 (Figure 2). Probit analysis showed that the lethal concentration (LT_{50}) recorded for *M. anisopliae* and *B. bassiana* was 3.966 x 10^7 and 1.721 x 10^7 spores/ml, respectively, while lethal time (LT_{50}) recorded for *M. anisopliae* and *B. bassiana* was 10.496 and 10.230 days, respectively (Table 7).

Table 7: *Lethal concentration (LC_{50}) and lethal time (LT_{50}).*

Lethal concentration (LC_{50})			
Entomo-pathogenic fungi (EPF)	n†	LC_{50}	Fiducial Limits 95 %
Metarhizium anisopliae	45	3.97 x 10^7	1.17 x 10^7 - 1.54 x 10^8
Beauveria bassiana	45	1.72 x 10^7	1.57 x 10^6 - 3.56 x 10^7
Lethal time (LT_{50})			
Entomo-pathogenic fungi (EPF)	n†	LT_{50} (days)	Fiducial Limits 95 %
Metarhizium anisopliae	45	10.49	8.28 - 16.08
Beauveria bassiana	45	10.23	7.11 - 21.69

† = Number of larvae used in bioassay.

Ovipositional preference

M. anisopliae and *B. bassiana* were evaluated against the ovipositional preference of tomato fruit worm, *H. armigera* on treated tomato plants in the laboratory. Experimental results showed that significant egg laying was recorded on treated tomato plants (P < 0.05; F =5.93). Comparison of each treatment with mean values and standard errors showed that the minimum mean egg laying i.e., 21.00 eggs was recorded in *Beauveria bassiana* @ 1×10^8 spores/ml followed by *M. anisopliae* @ 1×10^8 spores/ml (22.00 eggs.), *B. bassiana* @ 5×10^7 spores/ml (35.00 eggs), *B. bassiana* @ 1×10^7 spores/ml (49.00 eggs.), *M. anisopliae* @ 5×10^7 spores/ml (59.67 eggs) and

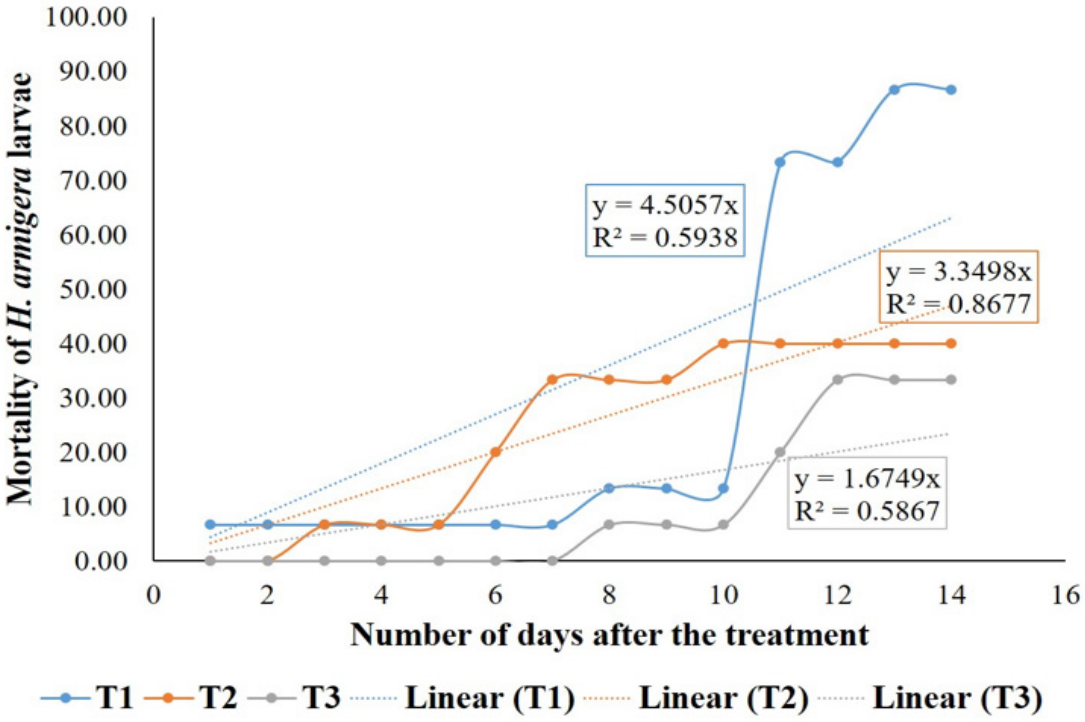

Figure 1: *Mortalityof H. armigera larvae as aresult on treated with M. anisopliae at different concertation i.e., T1 = M. anisopliae (1×10⁸ spores/ml); T2 = M. anisopliae (5×10⁷ spores/ml); T3 = M. anisopliae (1×10⁷ spores/ml).*

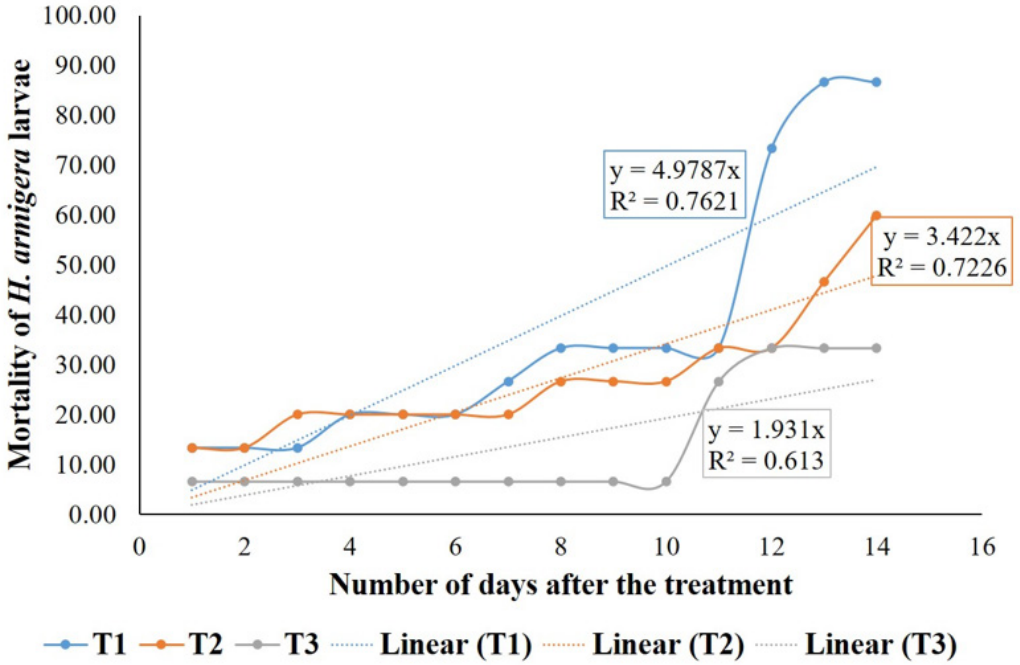

Figure 2: *Mortalityof H. armigera larvae as aresult on treated with B. bassiana at different concertation i.e., T1 = B. bassiana (1×10⁸ spores/ml); T2 = B. bassiana (5×10⁷ spores/ml); T3 = B. bassiana (1×10⁷ spores/ml).*

M. anisopliae @ 1×10⁷ spores/ml (77.00 eggs) while maximum mean egg laying was recorded in control treatment i.e., 103.33 eggs (Table 8).

Egg hatch rate (%)

M. anisopliae and *B. bassiana* were evaluated against egg hatching percentage of tomato fruit worm, *H. armigera* in laboratory. Experimental results showed the significant egg hatch rate of *H. armigera* under controlled conditions ($P < 0.05$; $F = 7.44$). Comparison of each treatment with mean values and standard errors. The result showed that the minimum mean egg hatch rate i.e., 49.58% of *H. armigera* was recorded in *B. bassiana* @ 1×10⁸ spores/ml followed by *B. bassiana* @ 5×10⁷ spores/ml (56.62%.), *M. anisopliae* @ 1×10⁸ spores/ml (70.37%.), *B. bassiana* @ 1×10⁷ spores/ml

(76.85%), *M. anisopliae* @ 5×10^7 spores/ml (79.64%.) and *M. anisopliae* @ 1×10^7 spores/ml (81.05%.) while maximum mean egg hatch rate was recorded in control treatment i.e., 86.30% (Table 9).

Table 8: *Mean table for ovipositional preference of H. armigera on tomato plants treated with different entomopathogenic treatments.*

Sr. No.	Treatment	Oviposition (Eggs) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	22.00 ± 7.21 d
T2	*M. anisopliae* (5 × 10^7 spores/ml)	59.67 ± 5.36 bc
T3	*M. anisopliae* (1 × 10^7 spores/ml)	77.00 ± 8.74 ab
T4	*B. bassiana* (1 x 10^8 spores/ml)	21.00 ± 6.56 d
T5	*B. bassiana* (5 × 10^7 spores/ml)	35.00 ± 15.09 cd
T6	*B. bassiana* (1 × 10^7 spores/ml)	49.00 ± 15.50 bcd
T7	Control (water)	103.33 ± 20.22 a
	LSD value	37.63

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Table 9: *Mean table for egg hatching rate of H. armigera on tomato plants treated with different concentrations of entomopathogenic fungi.*

Sr. No.	Treatment	Egg hatching (%) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	70.37 ± 2.45 bc
T2	*M. anisopliae* (5 × 10^7 spores/ml)	79.64 ± 3.88 ab
T3	*M. anisopliae* (1 × 10^7 spores/ml)	81.05 ± 7.25 ab
T4	*B. bassiana* (1 x 10^8 spores/ml)	49.58 ± 4.68 d
T5	*B. bassiana* (5 × 10^7 spores/ml)	56.62 ± 8.34 cd
T6	*B. bassiana* (1 × 10^7 spores/ml)	76.85 ± 2.29 ab
T7	Control (water)	86.30 ± 1.89 a
	LSD value	15.12

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Damage assessment (%)

M. anisopliae and *B. bassiana* were evaluated to check the percent damage assessment of tomato fruit worm, *H. armigera* in laboratory. Experimental results showed that the *H. armigera* larvae caused significant damage under controlled conditions (P < 0.05; F = 4.23). Comparison of each treatment with mean values and standard errors showed that the minimum mean damage assessment (%) of *H. armigera* i.e.,17.58% was recorded in *B. bassiana* @ 1×10^8 spores/ml followed by *M. anisopliae* @ 1×10^8 spores/ml (23.58%), *B. bassiana* @ 5×10^7 spores/ml (27.58%), *B. bassiana* @ 1×10^7 spores/ml (35.06%), *M. anisopliae* @ 5×10^7 spores/ml (36.57%.) and *M. anisopliae* @ 1×10^7 spores/ml (51.21%) while maximum mean damage assessment (%) was recorded in control treatment (water) i.e., 60.01% (Table 10).

Table 10: *Mean table for damage assessment (%) of H. armigera in different entomopathogenic treatments.*

Sr. No.	Treatment	Damage assessment (%) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	23.58 ± 2.00 cd
T2	*M. anisopliae* (5 × 10^7 spores/ml)	36.57 ± 1.93 b
T3	*M. anisopliae* (1 × 10^7 spores/ml)	51.21 ± 2.63 a
T4	*B. bassiana* (1 x 10^8 spores/ml)	17.58 ± 2.10 d
T5	*B. bassiana* (5 × 10^7 spores/ml)	27.58 ± 1.30 bc
T6	*B. bassiana* (1 × 10^7 spores/ml)	35.06 ± 6.20 b
T7	Control (water)	60.01 ± 3.55 a
	LSD value	9.72

Means within columns followed by the different lowercase letters are significantly different at P < 0.05.

Increase yield

M. anisopliae and *B. bassiana* were evaluated to check the increase yield (%) of tomato over control treatment. Experimental results showed the significant increase yield of tomato fruits over control under controlled conditions (P < 0.05; F = 22.3). Comparison of each treatment with mean values and standard errors showed that the minimum yield increase (%) of tomato fruits i.e., 26.04% was recorded in *B. bassiana* @ 1×10^7 spores/ml followed by *M. anisopliae* @ 1×10^7 spores/ml (27.18%), *M. anisopliae* @ 5×10^7 spores/ml (32.32%), *B. bassiana* @ 5×10^7 spores/ml (42.01%) and *M. anisopliae* @ 1×10^8 spores/ml (45.73%) while maximum yield increase (%) of tomato fruits was recorded in *B. bassiana* @ 1×10^8 spores/ml i.e., 54.40% over control treatment (Table 11).

Table 11: *Mean table for increase yield (%) of tomato in different entomopathogenic treatments.*

Sr. No.	Treatment	Increase yield (%) ± Std. error
T1	*M. anisopliae* (1 × 10^8 spores/ml)	45.73 ± 4.73 ab
T2	*M. anisopliae* (5 × 10^7 spores/ml)	32.32 ± 3.95 bc
T3	*M. anisopliae* (1 × 10^7 spores/ml)	27.18 ± 3.37 c
T4	*B. bassiana* (1 x 10^8 spores/ml)	54.40 ± 2.42 a
T5	*B. bassiana* (5 × 10^7 spores/ml)	42.01 ± 1.76 abc
T6	*B. bassiana* (1 × 10^7 spores/ml)	26.04 ± 6.27 c
	LSD value	16.91

Means within columns followed by the different lowercase letters are significantly different at P < 0.05

Discussion

The present research was conducted to evaluate the effect of *B. bassiana* and *M. anisopliae* against tomato fruit worm, *H. armigera* and results revealed that both fungi were virulent to *H. armigera*. Among the tested entomopathogenic fungi, *B. bassiana* was more virulent than the *M. anisopliae* under controlled conditions. In current study, the fastest mortality of *H. armigera* larvae was observed in petri dishes treated with *B. bassiana* at higher concentration i.e., 1×10^8 spores/ml. These results are align with the findings of Swathi *et al.* (2017), who reported that *B. bassiana* requires minimum time to kill *H. armigera* larvae compared to *M. anisopliae* under controlled conditions.

The results of the current experiment revealed that the highest mean corrected mortality of *H. armigera* larvae was recorded in *M. anisopliae* and *B. bassiana* at higher concentration. These findings are consistent with those of Douro *et al.* (2012), who reported maximum larval mortality of *H. armigera* with *B. bassiana* and *M. anisopliae* at higher concentrations. Furthermore, our results are similar to some extent with the observations of Ana *et al.* (2018).

In current study, the lowest mean pupal recovery, reduced pupal weight and absence of adult emergence were recorded in *B. bassiana* at high concentration of 1×10^8 spores/ml. Jarrahi and Safavi (2016) also reported the similar findings that *M. anisopliae* and *B. bassiana* exhibit virulent and adverse-effects on the biological parameters of *H. armigera* and causing substantial larval mortality.

Results of the current study indicated that the lowest LC_{50} and LT_{50} values were recorded for *B. bassiana* at higher concentration as compared to *M. anisopliae*. These findings are consistent with those of Swathi *et al.* (2017) who demonstrated that *B. bassiana* had lowest LC_{50} and LT_{50} values against *H. armigera* larvae under laboratory conditions. Several studies have reported the insecticidal activity of *B. bassiana* against *Rhynchophorus ferrugineus* (El Kichaoui *et al.*, 2017), *Plutella xylostella* (Sabbour and Sahab, 2005; Xia *et al.*, 2013), *Spodoptera exigua* (Wraight *et al.*, 2010), *H. armigera* (Douro *et al.*, 2012) and *Bemisia tabaci* (Mascarin *et al.*, 2013).

The mycelial outgrowth and conidial formation observed on the dead cadavers of treated larval instar of *H. armigera* confirmed that their mortality was occurred due to the application of entomopathogenic fungi. The results are align with the findings of Gabarty *et al.* (2014) and Lacey *et al.* (2015) who reported that fungal conidia is accountable for the infection by penetrating the host cuticle. Additionally, the yield data of the current experiment showed that the tomato plants treated with *B. bassiana* at higher concentration achieved the highest tomato yield increase over control compared to *M. anisopliae*. These findings are agreed with those of Patil *et al.* (2018), who demonstrated that tomato plants treated with *B. bassiana* produced higher tomato yields as compared to *M. anisopliae*.

Entomopathogenic fungi significantly affect the net reproductive rate of *H. armigera* moths. In our study, the highest egg laying (fecundity) and egg hatching percentage (fertility) were recorded in control treatment, where only water was sprayed. These results are similar to the findings of de Souza *et al.* (2020), who reported that the net reproductive rate (egg laying and hatching) of *H. armigera* was maximum in control treatment as compared to entomopathogenic fungal isolates such as *M. anisopliae* and *B. bassiana*.

Conclusions and Recommendations

It is concluded from the above experiments that both entomopathogenic fungi i.e., *B. bassiana* and *M. anisopliae* are highly effective in terms of pathogenicity against tomato fruit worm larvae, *H. armigera*. *Beauveria bassiana* at high concentration exhibited superior performance resulting in the earliest mortality, maximum corrected mortality, minimum pupal recovery and reduced adult emergence as compared to *M. anisopliae*. The lowest LC_{50} and LT_{50} values were also recoded for *B. bassiana* than *M. anisopliae*. Moreover, minimum fecundity and egg hatching percentage was recorded at higher concentrations of *B. bassiana* and *M. anisopliae*. Furthermore, maximum tomato yield (%) was also recorded in tomato plants treated with *B. bassiana* at higher concentration as compared to *M. anisopliae*. It is recommended that *B. bassiana* and *M. anisopliae* at a concentration of 1×10^8 spores/ml be utilized for the effective management of tomato fruit worm, *H. armigera* in vegetable crops.

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Novelty Statement

The novelty of this research lies in the evaluation of two entomopathogenic fungal base products, RACER® (*Beauveria bassiana*) and PACER® (*Metarhizium anisopliae Metarhizium anisopliae*) against *Helicoverpa armigera*, a major pest of tomato crops. This study not only explores their efficacy in reducing pest populations but also provides insights into environmentally friendly and sustainable alternatives to chemical insecticides. The findings contribute to integrated pest management (IPM) strategies, potentially offering new avenues for pest control that minimize environmental impact and pesticide resistance.

Authors Contribution

Abdul Basit: Reviewed the literature, conducted the experiments and wrote the initial manuscript draft.
Usman Khalique: Conceived the basic idea, conducted the experiments, statistical analysis and improved the manuscript draft.
Muhammad Salim: Collaborated in drafting the article.
Ahmad Ur Rahman Saljoqi: Supervised the study, helped in improving the basic idea and suggested improvements in the manuscript draft.

Conflict of interest
The authors declare that there is no conflict of interests regarding the publication of this article.

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