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Isolation, identification and virulence of indigenous entomopathogenic fungal strains against the peach-potato aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae), and the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

Sami Ullah[1], Abu Bakar Muhammad Raza[1], Mohamed Alkafafy[2], Samy Sayed[3], Muhammad Imran Hamid[4], Muhammad Zeeshan Majeed[1*], Muhammad Asam Riaz[1], Nevien M. Gaber[5] and Muhammad Asim[4]

Abstract

Background: As different biogeographic strains and isolates of entomopathogenic fungi vary in their genetic, enzymatic and pathogenic characteristics, this study assessed the virulence of 2 indigenous strains of *Beauveria bassiana* (Balsam) Vuillemin and *Metarhizium anisopliae* (Metschn.) Sorokin (Ascomycota, Hypocreales: Clavicipitaceae), isolated from naturally infected insect cadavers, against the 3rd instar nymphs of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) and 3rd instar larvae of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) using leaf-dip and larval-dip methods, respectively.

Results: Both fungal isolates exhibited considerable pathogenicity against *M. persicae* and *S. frugiperda*. Mortality in all bioassays was conidial concentration and exposure time dependent and increased significantly along with both factors ($R^2 = 0.86–0.99$ for *B. bassiana* and 0.82–0.94 for *M. anisopliae*). Moreover, *M. anisopliae* isolate appeared more virulent to *S. frugiperda* larvae than *B. bassiana* isolate, while the later fungal isolate was more lethal to *M. persicae* nymphs than the former one. At the highest conidial concentration (1.0×10^9 conidia/ml), *M. anisopliae* caused maximum mean mortality of *S. frugiperda* (88%) and *M. persicae* (65%) and *B. bassiana* exhibited maximum mean mortality of *S. frugiperda* (76%) and *M. persicae* (94%). Moreover, probit regression analyses showed LT_{50} values for *M. persicae* of 4.57 and 6.86 days at 1.0×10^9 conidia/ml for the isolates of *B. bassiana* and *M. anisopliae*, respectively, while LC_{50} values were 7.75×10^6 and 8.70×10^7 conidia/ml after 10th day of application, for the isolates of *B. bassiana* and *M. anisopliae*, respectively, against *M. persicae*. Similarly, LT_{50} values for *S. frugiperda* were 7.75 and 7.03 days for 1.0×10^9 conidia/ml concentration and LC_{50} values were 2.84×10^7 and 8.84×10^5 conidia/ml at 10th day data for the isolates of *B. bassiana* and *M. anisopliae*, respectively.

*Correspondence: zeeshan.majeed@uos.edu.pk
[1] Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan
Full list of author information is available at the end of the article

Conclusion: Overall study results demonstrated the effectiveness of *B. bassiana* and *M. anisopliae* against *M. persicae* and *S. frugiperda*, respectively. However, field evaluations of these indigenously isolated promising fungal strains against these insect pests.

Keywords: Entomopathogenic fungi, Indigenous isolates, *Beauveria bassiana*, *Metarhizium anisopliae*, *Spodoptera frugiperda*, *Myzus persicae*

Background

Almost all field and forage, fruit and vegetable crops and forest and ornamental plantations are attacked by a wide range of sucking and chewing insect pests (Lehmann et al. 2020). Aphids (Hemiptera; Aphididae) and armyworms (Lepidoptera; Noctuidae) are among the destructive and economically important insect pests throughout the world (Singh and Singh 2020).

Among aphids, the peach-potato aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is a highly polyphagous pest species that infests and damages about 400 plant species from 40 families around the globe including Pakistan (Hlaoui et al. 2019). Moreover, *M. persicae* vectors about 100 plant viruses. Similarly, fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is one of the economically important and notorious lepidopterous pests. It is native to tropical and subtropical Americas but has recently been dispersed to African and Asian countries including Pakistan (Gilal et al. 2020). It infests more than 80 agricultural crops, particularly rice, maize, cotton, millet, sorghum and sugarcane crops (De Groote et al. 2020).

Farmers in Pakistan rely primarily on synthetic pesticides to combat aphid and armyworm infestations on their crops. However, extensive use of broad-spectrum persistent synthetic chemicals has led to many issues such as insecticides resistance (Zhang et al. 2021), secondary pest outbreaks (Gross and Rosenheim 2011), eradication of beneficial fauna (Bueno et al. 2017), environmental contamination and human health hazards (Gomes et al. 2020). In view of these emerging ecological consequences of synthetic insecticides, it is imperative to seek out relatively safer and environment-friendly pest control options such as entomopathogenic fungi (EPF).

A wide range of EPF, particularly belonging to *Beauveria*, *Isaria*, *Lecanicillium* and *Metarhizium* genera, have been evidenced as potential biological control agents exhibiting significant virulence and pathogenicity against various insect pests. *M. persicae* and *S. frugiperda* are naturally infected by many species of EPF, some of which are very effective biocontrol agents (Firake and Behere 2020). The occurrence of such EPF naturally in an environment or agro-ecosystem indicates their potential role as biotic factors regulating insect pest populations in the field (Meyling and Eilenberg 2007).

Moreover, as EPF have a great genetic variation among their different biogeographic strains, the virulence and pathogenicity of different isolates to target insect pests may vary considerably due to their differential enzymatic and molecular characteristics (Maistrou et al. 2020). The present laboratory study aimed to isolate, identify and assess the virulence and entomopathogenicity of indigenous strains of EPF against the aphid *M. persicae* and the fall armyworm *S. frugiperda* as model mandibulate and haustellate phytophagous pests of economic importance, respectively.

Methods

Rearing of *S. frugiperda*

Late instar larvae of fall armyworm (*S. frugiperda*) were collected from the maize crop (*Zea mays* L.; hybrid cultivar Pioneer-32B33) grown in the farm area of Agriculture College, University of Sargodha (32°07′54" N; 72°41′35" E). The collected larvae were reared individually in a solitary manner in glass Petri plates (90 mm diameter) under controlled conditions (25 ± 2 °C, 65 ± 5% RH and 14-h: 10-h photoperiod). Larvae were fed on chickpea-based artificial diet prepared according to protocol of Jin et al. (2020) with slight modifications. Diet was changed daily until pupation and the newly formed pupae were placed on moistened filter paper discs lined in glass Petri plates (90 mm diameter). Newly emerged adults were shifted to rearing cages (30 × 30 × 30 cm; Bugdorm-I, Taiwan) for mating and egg lying and were provided with 10% honey solution soaked in cotton swabs as food. Egg clusters were shifted to glass Petri plates (90 mm diameter) on artificial diet. The culture of *S. frugiperda* was reared in the laboratory up to F_4 generation prior to their utilization in experimentation.

Rearing of *M. persicae*

Colonies of the peach-potato aphid (*M. persicae)* were randomly collected from the potato crop (*Solanum tuberosum* L.; white-skin cultivar Diamant) cultivated in the vicinity of Agriculture College, University of Sargodha (32°07′58" N; 72°41′32" E). This aphid population was reared on potted cabbage (*Brassica oleracea* L. var. *botrytis*) plants grown under controlled conditions (60 ± 10% RH and 27 ± 2 °C and 14-h: 10-h photoperiod). Old plants were replaced with new ones every week.

The culture of *M. persicae* was reared in the laboratory for several generations before its utilization in the bioassays. Healthy and active insect individuals were used in all bioassays.

Isolation of EPF from naturally dead insects

Cadavers of naturally infected insects were collected from the leaf litter around agricultural field banks and from the sideway areas of an irrigation canal junction (32°08′0.5" N; 72°40′46" E). These sites were selected because of known insect pest activities with no application of insecticides and fungicides for the last few months. Insect cadavers were collected and placed in zip-lock polythene bags and were brought to the laboratory for isolation of EPF. Field-collected insect cadavers, potentially having a fungal infection, were surface-sterilized with 0.5% aqueous solution of sodium hypochlorite, followed by 3 rinsing with sterile distilled water, and then were dried by placing and rolling them on sterile filter paper sheet. Subsequently, these larvae were placed in glass Petri plates (90 mm diameter) lined with moistened sterile filter paper discs and were incubated at 25 ± 2 °C to stimulate the conidial germination (Herlinda et al. 2008).

Fungal growth on insect cadavers was monitored on daily basis. When a fungal growth was observed, the conidia and hyphae were transferred to glass Petri plates (90 mm diameter) lined with potato dextrose agar (PDA) medium with the help of a sterile inoculation needle for purification and identification. Petri plates were incubated at 25 ± 2 °C and were inspected daily for fungal growth. At the point when more than one type of growth was developed on the same plate, they were isolated by sub-culturing. For further purification, small inoculum of fungal mycelia was cut out with a sterile inoculation needle and was moved to new Petri plates lined with Sabouraud dextrose agar (SDA) medium.

Identification of entomopathogenic fungi

Identification of isolated fungal strains was based on cultural and morphological features and was done by observing the growth pattern and colony formation of the fungal cultures. Slides were prepared from each isolate to identify its morpho-taxonomic characters using an inverted trinocular microscope (XDS-3, Optika SRL, Italy) by inspecting conidial structure and morphology according to the available literature and identification keys (Mongkolsamrit et al. 2020). Two cultures were identified as EPF: *Beauveria bassiana* (Balsam) Vuillemin and *Metarhizium anisopliae* (Metschn.) Sorokin (Ascomycota, Hypocreales: Clavicipitaceae) and were selected for further determination of their entomo-virulence against the model insect pests *i.e., M. persicae* and *S. frugiperda*.

Conidial suspensions preparation

Isolated cultures of *B. bassiana* and *M. anisopliae* were further mass cultured on Sabouraud dextrose agar yeast extract (SDAY) medium lined in glass Petri plates (90 mm diameter) incubated at 25 ± 2 °C. Conidial suspensions of both fungal isolates were prepared by harvesting their 15-day-old cultures with the help of a sterile inoculation loop and suspending them in 10 ml sterile double-distilled water in sterile vials containing 0.1% Tween-80 as surfactant. The solution was gently mixed and filtered through a three-layer sterile muslin cloth to eliminate other mycelial mass. Conidial concentrations of both fungal filtrates were determined by improved Neubauer's hemocytometer as described by Ibrahim et al. (2016). From the stock solution, serial dilutions were made by the concentrations of 1.0×10^9, 1.0×10^8, 1.0×10^7 and 1.0×10^6 conidia/ml and were stored in refrigerator at 4 °C until their downstream utilization in pathogenicity bioassays.

Pathogenicity assays

Bioassay of isolated EPF strains against *M. persicae*

Leaf-dip bioassay method as described by Nazir et al. (2019) was followed for determining the virulence of promising isolates of *B. bassiana* and *M. anisopliae* against *M. persicae*. In brief, leaf discs (50 mm diameter) were prepared from freshly clipped cabbage (*B. oleracea* var. *botrytis*) leaves and were dipped in each conidial concentration for 15 s. These treated leaf discs were placed on sterile filter paper sheet to remove excessive solution and were then shifted in sterile glass Petri plates (60 mm diameter) lined with 1.0% agar solution. In the control treatment, leaf discs were dipped in sterile double-distilled water containing 0.1% Tween-80 solution. Ten late 3rd instar nymphs of *M. persicae* were released on the treated leaf discs using sterile camel hair brush, and Petri plates were incubated under controlled conditions (65 ± 5% RH and 25 ± 2 °C). Mortality of exposed aphid individuals was recorded on 3rd, 5th, 7th and 10th day post-exposure. Dead aphid nymphs were removed and placed immediately on moistened filter paper discs lined in glass Petri plates (60 mm diameter) and were inspected daily for the development of fungal mycelia in order to confirm their fungus infection-induced death (Additional file 1: Fig. S1).

Bioassay of EPF isolates against *S. frugiperda*

The virulence of *B. bassiana* and *M. anisopliae* isolates was determined against *S. frugiperda* by larval-dip bioassay method according to a previously described protocol (Ramanujam et al. 2020). In brief, freshly molted (0–6 h old) 3rd instar larvae of *S. frugiperda* were immersed in

conidial concentrations for approximately 15 s. In control treatments, larvae were dipped in sterile double-distilled water having 0.1% Tween-80. Treated larvae were transferred in glass Petri plates (90 mm diameter) containing freshly cut leaves of cauliflower (*B. oleracea* var. *botrytis*) lined on 1.0% agar solution. Ten 3rd instar larvae of *S. frugiperda* were exposed in each Petri plate, and 5 replications were maintained for each treatment. Petri plates were incubated for 10 days under controlled conditions (27 ± 2 °C and 60 ± 5% RH). Leaves inside plates were changed every 2nd- or 3rd-day intervals. Larval mortality was recorded on 3rd, 5th, 7th and 10th day post-exposure. Dead larvae were removed and inspected for the fungal infection as described above (Additional file 1: Fig. S1).

Statistical analysis

All bioassays were conducted according to the completely randomized design (CRD) with 5 replications for each treatment. Using Statistix® Version 8.1 (Analytical Software, Tallahassee, FL), factorial analysis of variance (ANOVA) was run on the mortality data of insects taking conidial concentration and exposure time as factors. Treatment means were further compared by Tukey's honestly significant difference (HSD) post hoc test at standard level of probability ($\alpha = 0.05$). Probit regression analysis was performed to determine the median lethal concentration (LC_{50}) and time (LT_{50}) values using Polo Plus® software (LeOra Software, Parma, MO, USA, 2003).

Results

Virulence and pathogenicity of both indigenous isolates of *B. bassiana* and *M. anisopliae* were determined against laboratory-reared 3rd instar nymphs of *M. persicae* and 3rd instar larvae of *S. frugiperda* under laboratory conditions.

Mortality response of *M. persicae* to *B. bassiana* and *M. anisopliae* isolates

Factorial analysis of *M. persicae* bioassay revealed that there was a significant effect of both fungal concentration ($F_{3, 64} = 76.36$; $P \geq 0.001$ for *B. bassiana* and $F_{3, 64} = 31.53$; $P \geq 0.001$ for *M. anisopliae*) and time ($F_{3, 64} = 119.87$; $P \geq 0.01$ for *B. bassiana* and $F_{3, 64} = 71.65$; $P \geq 0.01$ for *M. anisopliae*) factors and their interactions ($F_{9, 64} = 5.14$; $P \geq 0.001$ for *B. bassiana* and $F_{9, 64} = 3.56$; $P \geq 0.001$ for *M. anisopliae*) on the mortality of *M. persicae* nymphs (Table 1).

Mean percent mortality of *M. persicae* nymphs was conidial concentration and time dependent as it increased along with the increase of conidial concentrations and exposure time. In case of *B. bassiana* isolate, aphid nymphal mortality increased significantly from day 3 to day 10 at all concentrations ($R^2 = 0.97$–0.99). Maximum nymphal mortality (94%) was exhibited by the highest concentration (1.0×10^9 conidia/ml) of *B. bassiana* recorded on 10th day of bioassay, while minimum aphid mortality (8–31%) was observed for the lowest concentrations (1.0×10^6 and 1.0×10^7 conidia/ml) on 3rd and 5th day of bioassay (Fig. 1). Similar trend of gradual and significant increase in the mortality of *M. persicae* nymphs was recorded for *M. anisopliae* isolate. Nymphal mortality increased significantly from day 3 to day 10 for all conidial concentrations ($R^2 = 0.86$–0.96). Maximum percent mortality (65%) was found at the highest conidial concentration (1.0×10^9 conidia/ml) on 10th day of bioassay, while the minimum aphid mortality values (6 – 24%) were recorded for low concentrations (1.0×10^6 and 1.0×10^7 conidia/ml) at 3rd and 5th day of bioassay (Fig. 2).

Probit regression analysis of data regarding percent corrected mortality of *M. persicae* nymphs revealed that *B. bassiana* was most virulent and fast-acting against 3rd instar nymphs of *M. persicae* than *M. anisopliae* isolate.

Table 1 Mean percent mortality of late 3rd instar nymphs of *Myzus persicae* exposed to different concentrations of indigenous isolates of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* under laboratory conditions

Source	DF	*Beauveria bassiana*				*Metarhizium anisopliae*			
		SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value
Time	3	24,500.0	8166.67	119.87	≥ 0.01	16,241.9	5413.96	71.65	≥ 0.01
Concentration	3	215,607.4	5202.47	76.36	≥ 0.001	7146.9	2382.30	31.53	≥ 0.001
Time × Concentration	9	3149.4	349.93	5.14	≥ 0.001	2419.6	268.85	3.56	≥ 0.001
Error	64	4360.2	68.13			4835.9	75.56		
Total	79	47,617.0				30,644.3			
Grand Mean		41.80				30.73			
CV		19.75				28.29			

$P \geq 0.001$ (highly significant) and $P \geq 0.01$ (significant); two-factor factorial ANOVA at $\alpha = 0.05$

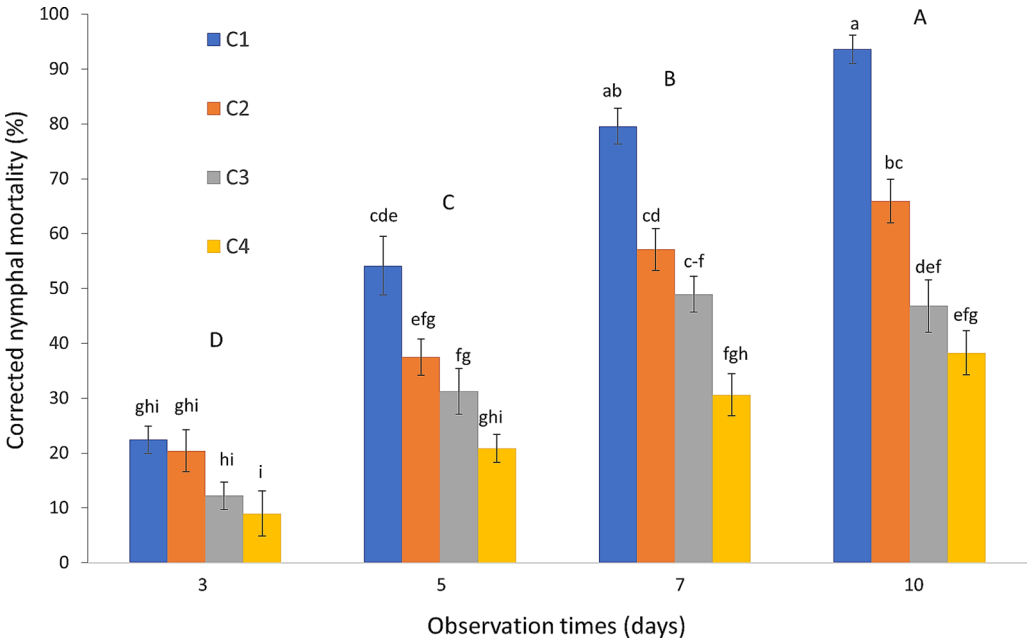

Fig. 1 Percent corrected mortality (mean ± S.E.) of late 3rd instar nymphs of peach-potato aphid *Myzus persicae* bioassayed against different concentrations of indigenously isolated strain of entomopathogenic fungus *Beauveria bassiana* under laboratory conditions. Concentrations C1–C4 correspond to 1.0×10^6–1.0×10^9 conidia/ml. Small letters at bar tops indicate significant difference among the concentrations, while capital letters indicate overall significant difference among the mortality at different time intervals (factorial ANOVA followed by Tukey's HSD test at $\alpha = 0.05$)

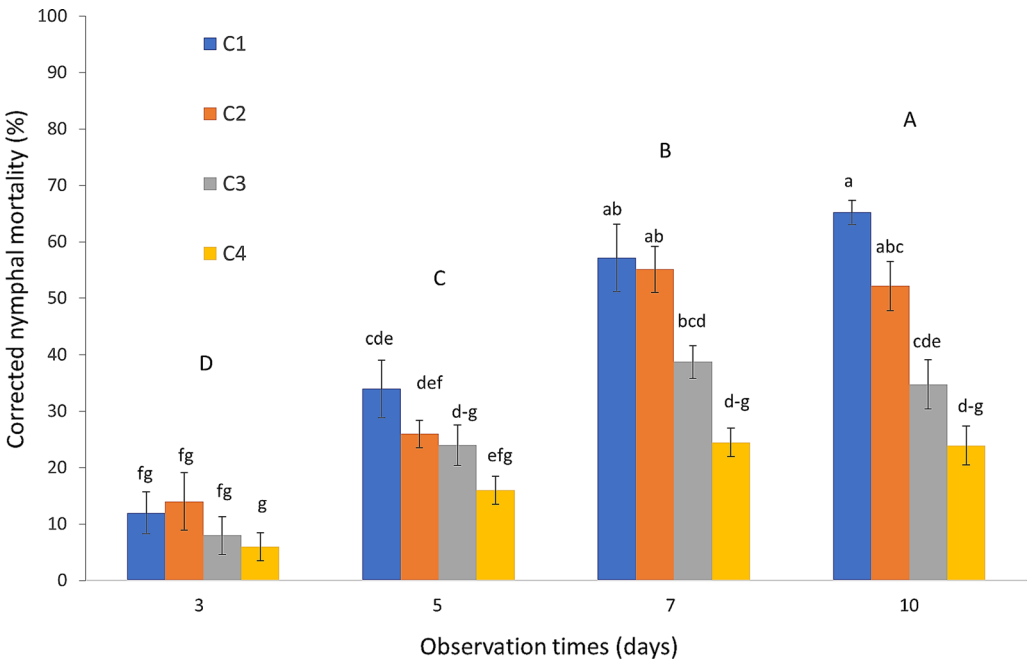

Fig. 2 Percent corrected mortality of late 3rd instar nymphs of peach-potato aphid *Myzus persicae* bioassayed against different concentrations of indigenously isolated strain of entomopathogenic fungus *Metarhizium anisopliae* under laboratory conditions. Concentrations C1–C4 correspond to 1.0×10^6–1.0×10^9 conidia/ml. Small letters at bar tops indicate significant difference among the concentrations, while capital letters indicate overall significant difference among the mortality at different time intervals (factorial ANOVA followed by Tukey's HSD test at $\alpha = 0.05$)

Median lethal time (LT_{50}) values were 4.57 and 6.48 days for 1.0×10^9 conidia/ml and were 6.86 and 7.88 days for 1.0×10^8 conidia/ml of *B. bassiana* and *M. anisopliae*, respectively (Table 2). Similarly, medial lethal concentration (LC_{50}) values of 1.67×10^7 and 7.75×10^6 conidia/ml at 7th day and 1.12×10^8 and 8.70×10^7 conidia/ml at 10th day were recorded for *B. bassiana* and *M. anisopliae*, respectively (Table 3).

Pathogenicity of *B. bassiana* and *M. anisopliae* isolates against *S. frugiperda* larvae

Factorial analysis revealed a significant effect of fungal concentrations ($F_{3,\ 64} = 31.63$; $P \geq 0.001$ for *B. bassiana* and $F_{3,\ 64} = 43.40$; $P \geq 0.001$ for *M. anisopliae*) and time ($F_{3,\ 64} = 300.63$; $P \geq 0.01$ for *B. bassiana* and $F_{3,\ 64} = 721.93$; $P \geq 0.01$ for *M. anisopliae*) factors and their interactions ($F_{9,\ 64} = 12.88$; $P \geq 0.001$ for *B. bassiana* and $F_{9,\ 64} = 7.44$; $P \geq 0.001$ for *M. anisopliae*) on the larval mortality of *S. frugiperda* (Table 4). For both fungal isolates, mean percent mortality of *S. frugiperda* larvae appeared in a time- and concentration-dependent manner as it increased along with the increase of exposure time and conidial concentration. For *B. bassiana* isolate, larval mortality increased significantly from day 3 to day 10 at all concentrations ($R^2 = 0.82$–0.93). Maximum percent mortality (76%) was observed by the highest concentration (1.0×10^9 conidia/ml) recorded

Table 2 Median lethal time (LT_{50}) values for the indigenous strains of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* bioassayed against late 3rd instar nymphs of peach-potato aphid *Myzus persicae* under laboratory conditions

EPF strains	Concentrations (conidia/ml)	LT_{50} (days)	Lower and upper 95% Fiducial Limits (days)	X^2 ($df = 30$)*	*P*-value	Slope ± S.E
Beauveria bassiana	1.0×10^9	4.571	4.220 – 4.909	68.748	≥ 0.001	4.353 ± 0.183
	1.0×10^8	6.480	5.797 – 7.386	59.180	≥ 0.001	4.353 ± 0.183
Metarhizium anisopliae	1.0×10^9	6.860	6.166 – 7.770	90.196	≥ 0.001	3.053 ± 0.167
	1.0×10^8	7.883	6.756 – 10.000	107.26	≥ 0.001	2.552 ± 0.171

*Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits

Table 3 Median lethal concentration (LC_{50}) values for the indigenous strains of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* bioassayed against late 3rd instar nymphs of peach-potato aphid *Myzus persicae* under laboratory conditions

EPF strains	Time (days)	LC_{50} (conidia/ml)	Lower and upper 95% Fiducial Limits (conidia/ml)	X^2 ($df = 30$)*	*P*-value	Slope ± S.E
Beauveria bassiana	7	1.674E + 07	8.543E + 06 — 3.069E + 07	57.638	≥ 0.001	0.417 ± 2.71
	10	7.752E + 06	3.043E + 06 — 1.580E + 07	108.124	≥ 0.001	0.547 ± 0.30
Metarhizium anisopliae	7	1.122E + 08	4.466E + 07 — 3.979E + 08	69.012	≥ 0.001	2.972 ± 0.26
	10	8.701E + 07	4.362E + 07 — 1.965E + 08	53.890	≥ 0.001	3.724 ± 0.28

*Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits

Table 4 Mean percent mortality of 3rd instar larvae of fall armyworm *Spodoptera frugiperda* exposed to different concentrations of indigenous isolates of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* under laboratory conditions

Source	DF	*Beauveria bassiana*				*Metarhizium anisopliae*			
		SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value
Time	3	32,130.0	10,710.0	300.63	≥ 0.001	54,145.0	18,048.3	721.93	≥ 0.001
Concentration	3	3380.0	1126.7	31.63	≥ 0.001	3255.0	1085.0	43.40	≥ 0.001
Time × Concentration	9	4130.0	458.9	12.88	≥ 0.001	1675.0	186.1	7.44	≥ 0.001
Error	64	2280.0	35.6			1600	25.0		
Total	79	41,920.0				60,675.0			
Grand Mean		24.00				28.75			
CV		24.87				17.39			

$P \geq 0.001$ (highly significant) and $P \geq 0.01$ (significant); two-factor factorial ANOVA at $\alpha = 0.05$

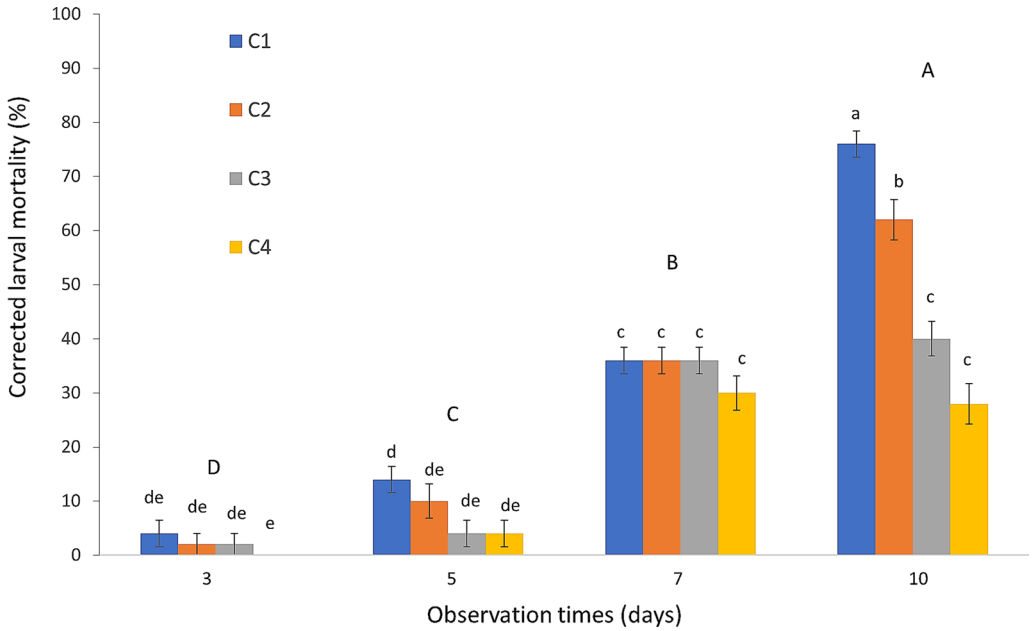

Fig. 3 Percent corrected mortality of 3rd instar larvae of fall armyworm *Spodoptera frugiperda* bioassayed against different concentrations of indigenously isolated strain of entomopathogenic fungus *Beauveria bassiana* under laboratory conditions. Concentrations C1–C4 correspond to 1.0×10^6–1.0×10^9 conidia/ml. Small letters at bar tops indicate significant difference among the concentrations, while capital letters indicate overall significant difference among the mortality at different time intervals (factorial ANOVA followed by Tukey's HSD test at $\alpha = 0.05$)

on 10th day of bioassay, while minimum mortality (2–4%) was observed for the lowest concentrations (1.0×10^6 and 1.0×10^7 conidia/ml) on 3rd and 5th day of bioassay (Fig. 3). For *M. anisopliae* isolate, larval mortality increased as well significantly from day 3 to day 10 at all concentrations ($R^2 = 0.91$–0.94). Maximum percent mortality (88%) was caused by the highest concentration (1.0×10^9 conidia/ml) recorded at 10th day of bioassay, while the minimum mortality values (0–6%) were observed for the lowest concentrations (1.0×10^6 and 1.0×10^7 conidia/ml) at 3rd day of bioassay (Fig. 4).

Probit analysis data revealed that *M. anisopliae* was most virulent and fast-acting against 3rd instar larvae of *S. frugiperda* than *B. bassiana* isolate. Median lethal time (LT_{50}) values were 7.75 and 8.71 days for 1.0×10^9 conidia/ml of *B. bassiana* and *M. anisopliae*, respectively, while these were 7.03 and 7.93 days for 1.0×10^8 conidia/ml of *B. bassiana* and *M. anisopliae*, respectively (Table 5). Similarly, LC_{50} values of 2.84×10^7 and 8.84×10^5 conidia/ml were recorded at 10th day for *B. bassiana* and *M. anisopliae*, respectively (Table 6).

Discussion

Contemporary issues of environmental contamination and health hazards being manifested by the extensive and recurrent use of highly persistent and hazardous synthetic insecticides necessitate looking for relatively safer and environment-friendly pest control options such as EPF which have been effective against a wide number of insect pest species (Litwin et al. 2020). However, these fungi exhibit considerable genetic variations among their biogeographic strains and the virulence and pathogenicity of different isolates to target insect pests may vary according to their differential enzymatic and molecular characteristics (Maistrou et al. 2020).

This laboratory work isolated, identified and assessed the virulence and pathogenicity of 2 promising indigenous strains of *B. bassiana* and *M. anisopliae* against *M. persicae* and *S. frugiperda* as a model mandibulate and haustellate phytophagous pests of economic importance. *B. bassiana* and *M. anisopliae* are ubiquitously found soil-born fungi capable of parasitizing a wide range of insect and mite pests (McGuire and Northfield 2020).

Bioassay results of aphids revealed that the indigenous isolate of *B. bassiana* was more pathogenic against 3rd instar nymphs exhibiting significantly a high mortality and minimum LT_{50} and LC_{50} values than those of *M. anisopliae*. These results are in line with the study of Bugti et al. (2018) in which four hemipteran pests including *M. persicae* were exposed to different conidial concentrations (1.0×10^2 to 6.75×10^5 conidia/mm^2) of a *B. bassiana* strain (Bb-202) and demonstrated that *B. bassiana* showed the highest pathogenicity to *M. persicae* and caused maximum mortality (100%) with LC_{50} and

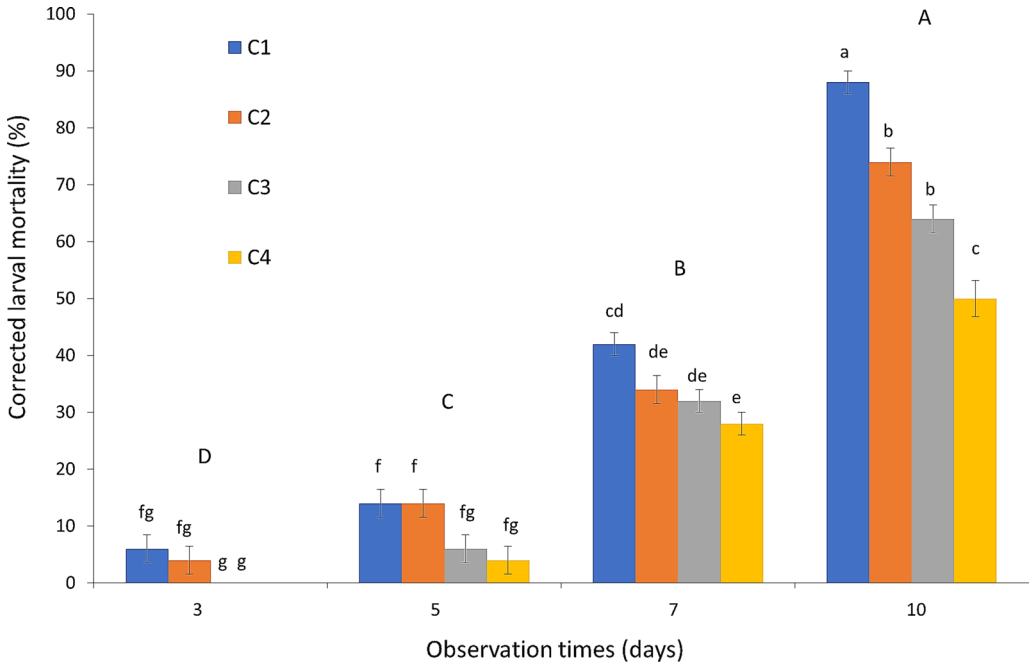

Fig. 4 Percent corrected mortality of 3rd instar larvae of fall armyworm *Spodoptera frugiperda* bioassayed against different concentrations of indigenously isolated strain of entomopathogenic fungus *Metarhizium anisopliae* under laboratory conditions. Concentrations C1–C4 correspond to 1.0×10^6–1.0×10^9 conidia/ml. Small letters at bar tops indicate significant difference among the concentrations, while capital letters indicate overall significant difference among the mortality at different time intervals (factorial ANOVA followed by Tukey's HSD test at $\alpha = 0.05$)

Table 5 Median lethal time (LT_{50}) values for the indigenous strains of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* bioassayed against 3rd instar larvae of fall armyworm *Spodoptera frugiperda* under laboratory conditions

EPF strains	Concentrations (conidia/ml)	LT_{50} (days)	Lower and upper 95% Fiducial limits (days)	X^2 ($df = 30$)*	*P*-value	Slope ± SE
Beauveria bassiana	1.0×10^9	7.750	7.142 – 8.540	111.67	≥ 0.001	5.056 ± 0.224
	1.0×10^8	8.705	7.919 – 9.905	95.075	≥ 0.001	4.721 ± 0.245
Metarhizium anisopliae	1.0×10^9	7.027	6.426 – 7.619	164.64	≥ 0.001	5.589 ± 0.225
	1.0×10^8	7.931	7.245 – 8.902	107.81	≥ 0.001	4.890 ± 0.232

*Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits

Table 6 Median lethal concentration (LC_{50}) values for the indigenous strains of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* bioassayed against 3rd instar larvae of fall armyworm *Spodoptera frugiperda* under laboratory conditions

EPF strains	Time (days)	LC_{50} (conidia/ml)	Lower and upper 95% Fiducial Limits (conidia/ml)	X^2 ($df = 30$)*	*P*-value	Slope ± SE
Beauveria bassiana	10 d	2.839E + 07	1.818E + 07 – 4.423E + 07	33.379	≥ 0.001	0.472 ± 0.029
Metarhizium anisopliae	10 d	8.843E + 05	2.973E + 05 – 1.901E + 06	25.809	≥ 0.001	0.356 ± 0.025

*Since the significance level is less than 0.150, a heterogeneity factor is used in the calculation of confidence limits

LT_{50} values of 6.7×10^4 conidia/ml and 5.2 to 8.24 days, respectively. Earlier, Kim et al. (2013) demonstrated the filtrates of *B. bassiana* were most pathogenic against *M. persicae* among 47 cultural filtrates of *Isaria*, *Lecanicillium*, *Beauveria* and *Cordyceps* spp. Similar results were revealed by Nazir et al. (2019) evaluating 3 strains of *B.*

bassiana and *Lecanicillium lecanii* against *M. persicae* adults. Also it was found that Bb-72 and Bb-252 strains of *B. bassiana* caused the highest mortality rate (95 and 91%, respectively) of *M. persicae* with minimum LC_{50} values (3.09×10^4 and 1.29×10^4 conidia/ml, respectively) and also showed mutual compatibility of these *B. bassiana* strains.

On the other hand, bioassays with armyworms revealed that the isolate of *M. anisopliae* was relatively more virulent against 3rd instar larvae of *S. frugiperda* and showed maximum mortality with minimum LT_{50} and LC_{50} values than those of *B. bassiana*. These results are in accordance with those of Cruz-Avalos et al. (2019) showed that among various strains of *B. bassiana*, *M. anisopliae* and *Nomuraea rileyi* isolated from soil samples and from naturally infected *S. frugiperda* larval cadavers, the isolates of *M. anisopliae*, particularly isolate *Ma*41, showed the highest larval mortality (100%) with LC_{50} value of 2.8×10^5 conidia/ml. A similar laboratory bioassay revealed 100% mortality of *S. frugiperda* larvae and eggs by a virulent strain of *M. anisopliae* with LT_{50} value of 2 to 3 days at 1.0×10^8 conidia/ml concentration (Lezama-Gutiérrez et al. 1996).

Many previous studies have demonstrated that *B. bassiana* and *M. anisopliae* are not only virulent and pathogenic against *M. persicae* and *S. frugiperda,* respectively, under laboratory bioassays (Nazir et al. 2019), but also have been effective under the field conditions and appeared promising options for sustainable management of *S. frugiperda* and other lepidopterous insect pests (Mwamburi, 2021) and against sucking insect pests including *M. persicae* (Dannon et al. 2020). In cage and field experiments, aqueous conidial suspensions of *B. bassiana* isolates (CG-864 and PL-63) were demonstrated to reduce the *M. persicae* population and infestation by 60 to 80% than the control (Filho et al. 2011).

However, the present findings are not in line with those of Montecalvo and Navasero (2021) who demonstrated that the virulence of *B. bassiana* and *M. anisopliae* varied according to different life stages of *S. frugiperda*. In this study, *B. bassiana* appeared more virulent to 1st than 6th larval instars with LC_{50} values of 0.06×10^8 to 9.43×10^8 conidia/ml, respectively, but LT_{50} values were 4.6 to 7.5 days, respectively. However, interestingly *M. anisopliae* isolate was more pathogenic to 3rd instar *S. frugiperda* larvae than *B. bassiana* although their difference was statistically non-significant. Moreover, the virulence of indigenous isolates may vary according to target insect pests. For instance, Gebremariam et al. (2021) showed that the indigenous Ethiopian isolates of *B. bassiana* were more pathogenic and lethal to *G. mellonella* than *M. anisopliae* isolates from the same soil samples.

Moreover, some studies have revealed the potential role of these EPF in phytopathogen antagonism, endophytism, rhizosphere colonization and in triggering the plant growth hormones (Ramos et al. 2020). Similarly, these insect parasitic fungi are also compatible with other pest control tactics including conventional and differential chemistry synthetic insecticides and along with other non-chemical control strategies (Quintela et al. 2013). For instance, both *B. bassiana* and *M. anisopliae* fungi have been shown compatibility with chlorpyrifos and spinosad (Rivero-Borja et al. 2018) and with pheromone traps (Akutse et al. 2020) against *S. frugiperda*.

Conclusions

It is concluded that the indigenous isolate of *M. anisopliae* was more virulent to *S. frugiperda* larvae than *B. bassiana* isolate, while the later fungal isolate appeared to be more lethal to *M. persicae* nymphs than the former one, exhibiting significant mortality and minimum LT_{50} and LC_{50} values. These results corroborate the effectiveness of different strains and isolates of *B. bassiana* and *M. anisopliae* against *M. persicae* and *S. frugiperda*, respectively, and advocate the significance of considering indigenous isolates of microbial biocontrol agents against native and exotic insect pests. However, field evaluations of these indigenously isolated promising fungal strains against these target insect pests and on their natural enemies constitute the future perspectives of this work.

Abbreviations

EPF: Entomopathogenic fungi; PDA: Potato dextrose agar; SDA: Sabouraud dextrose agar; SDAY: Sabouraud dextrose agar yeast extract; ANOVA: Analysis of variance; HSD: Honestly significant difference; LC_{50}: Median lethal concentration; LT_{50}: Median lethal time.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-021-00500-8.

Additional file 1. Infection and mycelial growth of *Beauveria bassiana* on 3rd instar nymph of *Myzus persicae*, and of *Metarhizium anisopliae* on 3rd instar larvae of *Spodoptera frugiperda*.

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Authors' contributions

MZM and ABMR conceived the idea and designed the study. SU and MA performed experimentation. MIH and NMG analyzed the data and prepared results. SU and MZM wrote the first draft of manuscript. MAR and MA technically proofread the manuscript. MZM and ABMR supervised the research work. SS and MZM provided technical and financial assistance for the study. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed in this work are available in the published manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
This study does not contain any individual person's data.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Entomology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan. [2]Department of Biotechnology, College of Science, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia. [3]Department of Science and Technology, University College-Ranyah, Taif University, B.O. Box 11099, Taif 21944, Saudi Arabia. [4]Department of Plant Pathology, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan. [5]Agricultural Research Center, Plant Protection Research Institute, Dokki, Giza 12311, Egypt.

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