Turkish Journal of Agriculture and Forestry

Volume 48 | Number 3 Article 11

6-6-2024

Eco-friendly management of Helicoverpa armigera using entomopathogenic fungi: a sustainable approach

HAFIZ HUSNAIN NAWAZ

GÜLŞAH KEKLİK

YAMIN BIBI

JAVED IQBAL

ATTIQ UR REHMAN

See next page for additional authors

Eco-friendly management of Helicoverpa armigera using entomopathogenic fungi: a sustainable approach

Authors
HAFIZ HUSNAIN NAWAZ, GÜLŞAH KEKLİK, YAMIN BIBI, JAVED IQBAL, ATTIQ UR REHMAN, MUBASHAR HUSSAIN, SHAHZAD TOUFEEQ, RASHID AZAD, KHALID ALI KHAN, ABID FARID, and ABDUL QAYYUM

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2024) 48: 470-489

doi:10.55730/1300-011X.3194

Eco-friendly management of *Helicoverpa armigera* using entomopathogenic fungi: a sustainable approach

Hafiz Husnain NAWAZ[1], Gülşah KEKLİK[2], Yamin BIBI[3], Javed IQBAL[4], Attiq ur REHMAN[5], Mubashar HUSSAIN[6], Shahzad TOUFEEQ[7], Rashid AZAD[8], Khalid Ali KHAN[9,10], Abid FARID[8], Abdul QAYYUM[11,*]

[1]Agricultural, Environmental and Food Sciences Faculty, Free University of Bozen-Bolzano, Bolzano, Italy
[2]Department of Animal Science, Faculty of Agriculture, Division of Biometry and Genetics, Çukurova University, Adana, Turkiye
[3]Department of Botany, Rawalpindi Women University, Rawalpindi, Pakistan
[4]Wheat Program, Barani Agricultural Research Institute, Chakwal, Pakistan
[5]Center of Excellence for Olive Research and Training, Barani Agricultural Research Institute, Chakwal, Pakistan
[6]Department of Zoology, University of Gujrat, Gujrat, Pakistan
[7]Key Laboratory of Insect Developmental and Evolutionary Biology, CAS Center for Excellence in Molecular Plant Sciences, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai, China
[8]Department of Entomology, The University of Haripur, Haripur, Pakistan
[9]Central Labs, King Khalid University, AlQura'a, Abha, Saudi Arabia
[10]Applied College, Center of Bee Research and its Products (CBRP), Unit of Bee Research and Honey Production, King Khalid University, Abha, Saudi Arabia
[11]Department of Agronomy, The University of Haripur, Haripur, Pakistan

Received: 15.12.2023 • **Accepted/Published Online:** 27.04.2024 • **Final Version:** 06.06.2024

Abstract: *Helicoverpa armigera* Hübner, 1808 (Noctuidae; Lepidoptera) is one of the major pest problems causing production constraints in both conventional and organic agricultural systems. Chemical and semichemical, along with biotechnological approaches, like the sterile insect technique, are used to manage pest populations. However, these methods raise environmental concerns. Therefore, the current study was undertaken to assess the biocontrol potential of the entomopathogenic fungal strains *Beauveria bassiana*, *Metarhizium anisopliae*, *Akanthomyces lecanii*, and *Purpureocillium lilacinus* against *H. armigera*. These strains, identified through morphological and molecular techniques, were evaluated for their effectiveness in controlling *H. armigera*. A total of eight fungal strains were identified as entomopathogens, including *B. bassiana* (Bb01, Bb02, Bb03, and Bb04), *Purpureocillium lilacinus* (Pl01, Pl02), *M. anisopliae* (Ma01), and *A. lecanii* (Vl01). The plate pathogenicity assay revealed that *B. bassiana* (Bb04) inflicted the highest mortality to *H. armigera* among all the entomopathogenic fungi studied. *B. bassiana* (Bb04) was selected for further studies based on its pathogenicity. BLAST analysis revealed a high similarity (99%–100%) between the internal transcribed spacer regions of the studied isolates. The total dry weight of mycelia was significantly higher than the control for 1% starch concentrated medium, followed by 1% sucrose, 1% dextrose, and 1% lactose, respectively. The results confirmed that the use of *B. bassiana* (Bb04) was an eco-friendly and sustainable approach that could replace chemical insecticides.

Key words: Entomopathogenic fungi, biocontrol, host–pathogen interactions, cotton pests, seed-based media, *H. armigera.*

1. Introduction

Humans have been using pesticides for different purposes since 20 BC (Faria and Wraight, 2007). Conventional chemical pesticide usage has increased manifold over the last few decades to meet the food requirements of an ever-increasing human population. This increased volume of pesticides has led to environmental contamination and posed threats to nontarget species (Kumar et al., 2019; Umar and Hussain, 2023). Reducing pesticide usage without compromising the yield and quality of agricultural production by encouraging biological control agents and implementing cultural practices can help protect the environment and conserve nontarget species (Rusch et al., 2010; Hussain et al., 2021). Biological control of insect pests has been achieved by using predators, parasitoids, and entomopathogenic fungi (Hoddle and Van Driesche, 2009; Labbé et al., 2009; Saldarriaga Ausique et al., 2017).

* Correspondence: aqayyum@uoh.edu.pk

Entomopathogenic fungi are naturally occurring organisms that cause minimal environmental damage. Their mode of action is quite complex, making resistance to the biopesticide exceedingly improbable. Many countries have approved these fungi as biopesticides (Mantzoukas and Eliopoulos, 2020). Entomopathogens have a specific mechanism for causing pathogenicity (Alford, 2000) and require air, water, carbon, nitrogen, and minerals for their growth. Their entomopathogenic ability against insects varies with environmental changes (Kin et al., 2017; Lydia et al., 2017) and depends on nutritional molecules, including carbohydrates, organic acids, amino acids, and vitamins, in proper proportions. A lack or excess of these substances may impact the survival, mycelial growth, sporulation, and spore germination of the fungus. Several microbial pathogens are involved and systematically studied in biological control for their beneficial purposes. In biological control agents, entomopathogenic fungi are more diverse and have greater potential for causing disease incidence compared to other natural enemies of insects pests (Waheed et al., 2012; Pérez-González and Sánchez-Peña, 2017; Becher et al., 2018).

Fungi have showed promise as a selective insecticide in the past (Lydia et al., 2017). *Metarhizium* spp., *Beauveria* spp., *Akanthomyces lecanii*, *Beauveria bassiana*, and *Metarhizium anisopliae* are amongst the most promising entomopathogenic fungi, exhibiting an extensive susceptible range in crop pests. *B. bassiana* usually infects insects on the upper side of the soil surface, while *M. anisopliae* is particularly recommended for insects residing within the soil plane (Mahr et al., 2001; Ugine et al., 2013; Komaki et al., 2017; Mohammed and Hatcher, 2017; Sabbahi et al., 2017). The anamorphic entomopathogen species *B. bassiana* and *M. anisopliae* from Hypocreales are innate predators of different insect pests (Alford, 2000; Ashraf et al., 2017; Kryukov et al., 2018). *B. bassiana* and related insect-pathogenic fungi have been extensively used as biocontrol agents against a broad range of insect species. However, their field performance can vary, and they may exhibit low killing ability (Lovett and St. Leger, 2018). Moreover, abiotic factors such as environmental stresses and UV light also affect *B. bassiana* efficacy (Méndez-González et al., 2022). Therefore, it is vital to assess the virulence of *B. bassiana* and other entomopathogenic fungi in the laboratory experiments before using them as large-scale biocontrol agents.

In this study, we aimed to evaluate different entomopathogenic fungi strains tested individually in the lab to assess their potential as sustainable biocontrol agent against *H. armigera.* We emphasize (i) different ecology niches of these strains at diverse locations; (ii) representing morphological and culture characters of these strains; (iii) virulence efficacy of these strains against *H. armigera*; and (iv) molecular identification and characterization of collected strains. These results will provide a better understanding of the identification of these strains and their potential as biocontrol agents.

2. Materials and methods

2.1 Isolation of fungi

A survey of entomopathogens was conducted in several agro-climatic areas of Punjab province (Pakistan) from May 2018 to September 2019. Fungal-infected insects and soil samples from different crops were also collected (Table 1). The insect samples were treated with sodium hypochlorite (2%) followed by washing with sterilized water. The samples were then incubated at 28 ± 1 °C for 5 days to achieve fungal growth (Ownley et al., 2010). As the growth of mycelium became visible, certain hyphae or conidia were transferred to a fresh Potato Dextrose Agar (PDA) medium to obtain a pure culture. The plates were kept at 28 ± 1 °C for 5 to 21 days. For further experiments, pure isolates were stored at 4 °C. Thus, a total of 37 fungal strains were successfully cultured.

2.2. Morphological studies

Twelve-day-old colonies of all 37 cultivated fungal strains were examined for morphological identification of the fungal strains for observed characteristics of hyphae, conidiophores, and conidia using a Carl Zeiss optical microscope at 400× and 1000× magnifications (Mehta et al., 2012; Ferrigo et al., 2017). Color on the top and bottom sides of the plates, form, surface, edge, consistency, and inclination were also noted for each colony. The mean and standard deviation of the reproductive structures, as determined by microscopic measures, were computed. After identification, the fungal strains were submitted to the Fungal Culture Bank, University of the Punjab (Pakistan).

2.3. In vitro pathogenicity assays

Helicoverpa armigera were brought to the laboratory from the Entomology Department, University of the Punjab, Lahore, Pakistan. Fungal isolates were cultured in 100 mL of potato dextrose broth in Erlenmeyer flasks for 10 days at 28 ± 1 °C under dark conditions. After 10 days, a cheesecloth filter was used to remove mycelium from the media. The blastospore-rich medium was transferred to 15-mL centrifuge tubes and centrifuged in 2000 rcf for 5 min to extract the blasts. Using Tween 80 0.01% (v/v), the pellet was resuspended and centrifuged again to remove any remaining culture media, and the supernatant was discarded. At 400× magnification in a Carl Zeiss optical microscope, blastopore suspensions were measured using a hemocytometer, and the concentrations of 3×10^7 mL^{-1} propagules were adjusted for each bioassay.

Table 1. Different strains of entomopathogenic fungi isolated from different insects in different regions of Pakistan.

Entomopathogenic fungi	Strain	Source	Crop	Place of collection			
				Location	GPS		Soil type
					Latitude	Longitude	
Beauveria bassiana	Bb01	Plant Hopper	Cotton	Lahore	31.582045	74.329376	Silt-loam
Beauveria bassiana	Bb02	Grey weevil	Cotton	Multan	30.181459	71.492157	Clayey
Beauveria bassiana	Bb03	Red cotton bug	Cotton	RYK	57.883	29.233	Loamy
Beauveria bassiana	Bb04	Jassid	Cotton	Sadiqabad	28.310350	70.127403	Clayey and sandy
Metarhizium anisopliae	Ma01	Grey weevil	Cotton	Lahore	31.245659	74.212891	Sandy
Paecilomyces lilacinus	Pl01	Spotted bollworm	Cotton	Multan	30.20146	71.48059	Calcareous
Paecilomyces lilacinus	Pl02	Grey weevil	Cotton	Sialkot	32.497223	74.536110	Clay loam
Lecanicillium lecanii	Vl01	Moth	Cotton	Lahore	31.621113	74.282364	Silt-loam

We inoculated (without spreading) 20 µL of each solution into the center of Petri plates filled with PDA to examine survival and viability of fungal propagules. It was maintained in the dark at 28 ± 1 °C for 3 to 4 days to monitor germination. The prepared fungal blastopore concentration was applied to *H. armigera* adults with a sprayer in three replications. Each replication had 50 larvae, and adults were also exposed to each entomopathogenic fungi raised on insect rearing plates at room temperature with 70% humidity. On the 5th day, the mortality percentage of each larval cohort was analyzed (Mahr et al., 2001; Sugimoto et al., 2001; Husnain et al., 2014).

2.4. Molecular studies

2.4.1. DNA isolation and purification

Liquid cultures of the Entomopathogenic fungal isolates were grown in Erlenmeyer flasks at 28 ± 1 °C in potato dextrose broth for 10 days and kept in dark conditions. To extract and purify the DNA, we used the E.Z.N.A. Fungal DNA Mini Kit (Omega Bio-tek, Inc. Norcross, GA). To assess the purity and quality of the DNA, 0.8% (w/v) agarose gel electrophoresis was used. The gel was stained with ethidium bromide, and the DNA bands were visible under UV light. We stored the extracted DNA at –20 °C until it was needed.

2.4.2. PCR amplification

The molecular identification of the entomopathogenic fungal isolates was performed, and the internal transcribed spacer (ITS) region was amplified (Hung and Posta, 2017). ITS region for *B. bassiana* (Accession No: KT310219.1), *M. anisopliae* (Accession No: MK005254.1), *A. lecanii* (Accession No: FJ515770.1), and *P. lilacinus* (Accession No: MN242828.1) sequences previously uploaded to GenBank were used as reference sequences and to design new primers for previously described regions. Primer pairs ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) for *B. bassiana*, ITS4 (TCCTCCGCTTATTGATATGC) and ITS5 (GGAAGTAAAAGTCGTAACAAGG) for *A. lecanii*, ITS1 (TCCGTAGGT GAACCTGCGG) and ITS4 (TCCTCCGCTTATT GATGC) for *P. lilacinus*, and ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) for *M. anisopliae* were used to amplify the ITS region (Hung and Posta, 2017) (BGI Genomics InL, Shenzhen, China). With these primers, the expected product size for the ITS region is 600 bp. One microliter of genomic DNA, 1 µL of forwarding primer, 1 µL of reverse primer, 10 µL of deionized water, and 12.5 µL of 2X Eco Taq Supermix were used in a 25.5 µL PCR reaction (Thermo Fisher Scientific Inc., Waltham, MA, USA). A GeneAmp 9700 DNA thermal cycler was used for DNA amplification (PerkinElmer Inc., Waltham, MA, USA). The first denaturation took place at 95 °C for 1 min, and then there were 36 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min. During the final extension, 72 °C was maintained for 10 min. We analyzed PCR products using an agarose gel with a 0.8% (w/v) concentration. Molecular weight standards were determined using a 2000 bp DNA ladder (Sigma-Aldrich Co., St. Louis, MO, USA). Staining the gel with ethidium bromide was followed by observation under UV light and photography. The PCR products were then cleaned with an E.N.Z.A Gel Extraction Kit (Omega Bio-tek, Inc., Norcross, GA, USA) and sequenced with Sanger sequencing (BGI Genomics InL, Shenzhen, China) (Nawaz et al., 2018).

The MEGAX software program was used for alignment and sequence comparison tests (Tamura et al., 2013). The ITS region of the isolated strains of entomopathogenic fungi was compared with previously submitted sequences of entomopathogenic fungi (*B. bassiana*, *M. anisopliae*, *A. lecanii*, and *P. lilacinus*) at the GenBank database (Table 2).

2.4.3. DNA modeling and phylogenetic analysis

The consensus sequence was assembled using MEGAX software. For comparative purposes, closely related DNA sequences of the ITS region of *B. bassiana*, *M. anisopliae*, *A. lecanii*, and *P. lilacinus* were obtained from GenBank, and sequence alignment was performed using the

Table 2. NCBI GenBank accession numbers of isolates belonging to the entomopathogenic fungi that were used for comparing the variability of the ITS region with isolated entomopathogenic strains.

Entomopathogenic fungi	Strain	Origin	ITS
Beauveria Bassiana	IIFT-B8	Cuba	KT310219
Beauveria Bassiana	IIFT-B12	Cuba	KT310221
Beauveria Bassiana	IHBF15	India	MT111138
Beauveria Bassiana	CICR-RSS-0058	India	MK680181
Beauveria Bassiana	BbGD 48	China	MH483696
Beauveria Bassiana	IIFT-B10	Cuba	KT310220
Beauveria Bassiana	Bb69/3	Türkiye	KP862996
Beauveria Bassiana	BbKm-1	Türkiye	MT441868
Beauveria Bassiana	JL005	China	MH020635
Beauveria Bassiana	BbGD19	China	MH483665
Beauveria Bassiana	BbGD08	China	MH483652
Beauveria Bassiana	F19-N	Mongolia	MG640376
Beauveria Bassiana	SHU.M.161	China	KU158472
Beauveria Bassiana	SHU.M.058	China	KU158437
Beauveria Bassiana	SHU.M.017	China	KU158425
Beauveria Bassiana	I13	Iran	MZ956768
Beauveria Bassiana	YK26	Türkiye	MZ781311
Beauveria Bassiana	EABb04_01tip	Spain	KC753382
Beauveria Bassiana	DTG-54	Türkiye	MW534279
Beauveria Bassiana	SASRI BB444	South Africa	JX110368
Beauveria Bassiana	B4 18S	Mexico	GU189515
Beauveria spp.	JS-2009a	Canada	GQ354257
Beauveria Bassiana	EABb 04/01-Tip	Spain	DQ364698
Beauveria Bassiana	BbL_1	Lebanon	MT533246
Beauveria Bassiana	BbFn-2	Türkiye	MT441874
Beauveria Bassiana	JEF-462	South Korea	MN122426
Beauveria Bassiana	BbGX22A02	China	MH483871
Beauveria Bassiana	BbGD67	China	MH483713
Beauveria Bassiana	BbGD20	China	MH483667
Beauveria Bassiana	TMSL74C	Portugal	LT220531
Beauveria Bassiana	TMSL142	Portugal	LT220530
Beauveria Bassiana	TMCR13	Portugal	LT220529
Beauveria Bassiana	TMCR05	Portugal	LT220528
Beauveria Bassiana	TM1613	Portugal	LT220527
Beauveria Bassiana	TM1537A	Portugal	LT220526
Beauveria Bassiana	TM1306	Portugal	LT220525
Beauveria Bassiana	GMSL19	Portugal	LT220524
Beauveria spp.	GMSL117	Portugal	LT220523
Beauveria Bassiana	GMSL116	Portugal	LT220522
Beauveria Bassiana	GMSL11	Portugal	LT220521
Beauveria Bassiana	GMSL106	Portugal	LT220520
Beauveria Bassiana	GMSL07	Portugal	LT220519
Beauveria Bassiana	GMGJ93A	Portugal	LT220517
Beauveria Bassiana	GMGJ80	Portugal	LT220516
Beauveria Bassiana	GMGJ75A	Portugal	LT220515
Beauveria Bassiana	GMCR67	Portugal	LT220514

Table 2. (Continued.)

Beauveria Bassiana	GMCR62	Portugal	LT220513
Beauveria Bassiana	GMCR56	Portugal	LT220512
Beauveria Bassiana	GMCR51	Portugal	LT220511
Beauveria Bassiana	PYCC 8681	Portugal	OK094889
Beauveria Bassiana	YK23	China	MZ781314
Beauveria Bassiana	2773	Spain	KC753391
Beauveria Bassiana	SC33B07	China	MW113401
Beauveria spp.	CauBb35	India	MW633024
Beauveria Bassiana	CauBb53	India	MW627305
Beauveria Bassiana	GXTR1010	Iran	JQ999971
Beauveria Bassiana	F-HY002-1A	China	MN252575
Beauveria Bassiana	BbFn-1	Türkiye	MT441873
Beauveria Bassiana	Bb20	Türkiye	KP862976
Beauveria Bassiana	Aydinsoke	Türkiye	KP862967
Beauveria Bassiana	K2TG-94	Türkiye	MW534280
Beauveria Bassiana	KPA-89	Türkiye	MW534278
Beauveria Bassiana	EF_395	China	MT529044
Beauveria Bassiana	EF_113	China	MT528762
Beauveria Bassiana	EF_107	China	MT528756
Beauveria Bassiana	EF_71	China	MT528720
Beauveria Bassiana	EF_59	China	MT528708
Beauveria Bassiana	BB04	Pakistan	OL825010
Beauveria Bassiana	BB03	Pakistan	OL825009
Beauveria spp.	BB02	Pakistan	OL825008
Beauveria Bassiana	BB01	Pakistan	OL825007
Beauveria Bassiana	LY2	China	MZ262366
Beauveria Bassiana	n67	China	MZ356505
Beauveria Bassiana	WICC B208	Malaysia	OK036438
Beauveria Bassiana	CBS 325.67	Malaysia	MH858984
Beauveria Bassiana	TF27	China	MN515360
Beauveria Bassiana	B-Bot	China	MK862360
Beauveria Bassiana	BbSr-1	China	MT441882
Beauveria Bassiana	EF_8	China	MT528657
Beauveria Bassiana	EF_16	China	MT528665
Beauveria Bassiana	EF_17	China	MT528666
Beauveria Bassiana	EF_18	China	MT528667
Beauveria Bassiana	EF_24	China	MT528673
Metarhizium anisopliae	F-1	China	MF467274
Metarhizium anisopliae	Ma130	Mexico	JN256674
Metarhizium anisopliae	Ma129	Mexico	JN256673
Metarhizium anisopliae	XL501	China	GU909512
Metarhizium anisopliae	LRC 148	Australia	EU307893
Metarhizium anisopliae	DhMz3R	India	KU983783
Metarhizium anisopliae	ArMz2S	India	KU983775
Metarhizium anisopliae	NRCBMaEPF54	India	MT114694
Metarhizium anisopliae	CNHE	China	FJ545294
Metarhizium anisopliae	SBI-Ma16	India	MZ081556
Metarhizium anisopliae	LSNM3	China	FJ545313

Table 2. (Continued.)

Metarhizium anisopliae	ARSEF 1009	China	HM055446
Metarhizium anisopliae	NRCBMaEPF40	India	MT110992
Metarhizium anisopliae	NRCBMaEPF18	India	MN888763
Metarhizium anisopliae	M38	Costa Rica	MG786742
Metarhizium anisopliae	OEMC3	Costa Rica	KY053455
Metarhizium anisopliae	ArMz4R	India	KU983800
Metarhizium anisopliae	ArMz1S	India	KU983774
Metarhizium anisopliae	CNHB3	China	FJ545293
Metarhizium anisopliae	CNGU3	China	FJ545289
Metarhizium spp.	V1008	Greece	LR792756
Metarhizium spp.	ERL 700	Greece	LR792750
Metarhizium anisopliae	Ma01	Pakistan	OL839175
Metarhizium anisopliae	88173	Thailand	MK005254
Metarhizium anisopliae	NRCBMaEPF50	India	MT111128
Metarhizium spp.	29AL	Costa Rica	MT005747
Metarhizium anisopliae	NRCB Ma EPF-16	India	MK834813
Metarhizium spp.	M29	Costa Rica	MG786740
Metarhizium anisopliae	Ma68	Mexico	JN256675
Metarhizium anisopliae	VL001	Vietnam	EU530667
Metarhizium anisopliae	VL001/125	Vietnam	EU530666
Metarhizium anisopliae	NRCBMaEPF48	India	MT111119
Metarhizium anisopliae	NRCBMaEPF42	India	MT110996
Metarhizium anisopliae	NRCBMaEPF6	India	MN892391
Metarhizium anisopliae	NRCBMaEPF17	India	MN888761
Metarhizium spp.	30U	Costa Rica	MT005748
Metarhizium anisopliae	NRCBMaEPF10	India	MN892393
Metarhizium anisopliae	CBS 218.29	Netherlands	MH855048
Metarhizium spp.	EMP4	India	KY408033
Metarhizium anisopliae	WnMz2S	India	KU983787
Metarhizium anisopliae	SBI-Ma16	India	MZ081556
Metarhizium anisopliae	NRCBMaEPF53	India	MT114688
Metarhizium anisopliae	NRCBMaEPF47	India	MT111118
Metarhizium anisopliae	NRCBMaEPF40	India	MT110992
Metarhizium anisopliae	NRCBMaEPF12	India	MN892390
Metarhizium anisopliae	NRCBMaEPF18	India	MN888763
Metarhizium spp.	M9	Costa Rica	MG786736
Metarhizium spp.	M6	Costa Rica	MG786733
Metarhizium anisopliae	NRCBMaEPF24	India	MN893383
Metarhizium anisopliae	AUMC5130	Egypt	MN710409
Metarhizium anisopliae	NRCB Ma EPF-24	India	MK836090
Metarhizium anisopliae	NRCB Ma EPF-09	India	MK834805
Metarhizium anisopliae	NRCBMaEPF58	India	MT111550
Metarhizium anisopliae	NRCBMaEPF45	India	MT111116
Metarhizium anisopliae	NRCBMaEPF43	India	MT111094
Metarhizium spp.	YH31	China	MN602855
M. guizhouense	ARSEF:14330	China	MN255811
Metarhizium spp.	M38	Costa Rica	MG786742
Metarhizium spp.	OEMC3	Costa Rica	KY053455

Table 2. (Continued.)

M. robertsii	ArMz4R	India	KU983800
M. guizhouense	RIFA 86A	USA	KF624796
M. pingshaense	4342	Greece	LR792764
M. guizhouense	4321	Greece	LR792761
M. pinghaense	ARSEF 2162	China	M055447
M. guizhouense	ARSEF 977	China	HM055445
M. guizhouense	ARSEF 703	China	HM055444
M. anisopliae	NHJ6195	China	AY646393
M. anisopliae	NRCBMaEPF41	India	MT110994
Metarhizium Spp.	strain R	Greece	LR792770
M. robertsii	strain 727	Greece	LR792768
A. lecanii	ICAL-6 18S	Spain	FJ515770
A. lecanii	ICAL-4 18S	Spain	FJ515768
Lecanicillium spp.	CH113	Chile	MH231248
E. typhina	AL Ep1	Ireland	MZ509315
L. longisporum	CBS 384.35	Czech Republic	MH855713
L. attenuatum	xinmin li:V6	China	MH231313
A. lecanii	ACB30112	China	MW465671
A. lecanii	strain Z3	China	MW165534
L. attenuatum	NBRC 103236	India	AB378511
L. attenuatum	KYK00039	Japan	AB378508
L. attenuatum	KYK00134	Japan	B378515
L. muscarius	CBS 318.70I	Czech Republic	MH859692
L. muscarius	CBS 318.70A	Czech Republic	MH859685
L. attenuatum	NBRC 103238	India	AB378512
L. muscarium	B2 V	USA	MF872368
A. lecanii	AL Ak2	Ireland	MZ509283
L. attenuatum	CGMCC5328	China	JQ901939
A. lecanii	Vl01	Pakistan	OL827536
A. lecanii	ICAL-1	Spain	FJ515765
Cordyceps confragosa	Tn-29	Pakistan	LT626265
Cordyceps confragosa	Tn-27	Pakistan	LT626263
A. lecanii	ICAL-7	Spain	FJ515771
Cordyceps confragosa	Tn-30	Pakistan	LT626264
L. longisporum	CBS 384.35	Czech Republic	MH855713
A. lecanii	KYK00324	Japan	AB378513
A. lecanii	NBRC 103236	India	AB378511
A. lecanii	NBRC 103235	India	AB378510
A. lecanii	KYK00039	Japan	AB378508
A. lecanii	KYK00034	Japan	AB378507
A. lecanii	KYK00134	Japan	AB378515
A. lecanii	KYK00326	Japan	AB378514
A. lecanii	NBRC 32311	Japan	AB111495
L. muscarium	C13	USA	MF872370
L. attenuatum	NBRC 103237	Japan	AB360369
L. muscarium	B2_V	USA	MF872368
L. attenuatum	CGMCC5328	China	JQ901939
Paecilomyces lilacinus	Pl01	Pakistan	OL827568

Table 2. (Continued.)

P. lilacinus	Pl02	Pakistan	OL827569
P. lilacinus	F129	Pakistan	MN242828
P. lilacinus	SR50	China	KX009140
P. lilacinus	xz16	China	KJ935014
P. lilacinus	RIZ4-4	Nigeria	MW260105
P. lilacinus	NKCM1001	Japan	AB244777
P. lilacinus	PU16Z12577	China	MT254824
P. lilacinus	CES3	India	MN173144
P. lilacinus	SF_327	Mexico	MT529603
P. lilacinus	001JFC	Mexico	KR025540
P. lilacinus	C13	USA	KJ938575
P. lilacinus	Kw596W	KUWAIT	FR751342
P. lilacinus	SY45B-a	Canada	HM242264
P. lilacinus	LTBF 007-1	Brazil	GQ229080
P. lilacinus	UWFP 853	USA	AY213668
P. lilacinus	Mal02-Mod	Italy	MH426603
P. lilacinus	DSM100329_DF58_RLCS20	Germany	MT453285
Purpureocillium Spp.	GFR22	China	MT447527
P. lilacinus	BCC<THA>:86507	Thailand	MH430168
Purpureocillium Spp.	MCM_HumanF8-1	Thailand	AB915809
P. lilacinus	B3A	Canada	HM242262
P. lilacinus	MY683	Thailand	GU980015
P. lilacinus	PUtp11	China	MT279299
P. lilacinus	IIPRPl-2	India	MK713625
P. lilacinus	R38	Kenya	MT420635
P. lilacinus	131	Brazil	MH137675
P. lilacinus	YCG1	China	KM268709
P. lilacinus	MY2861	Thailand	GU980027
P. lilacinus	3-F20	China	MW081316
P. lilacinus	BCC 2012	Thailand	EU828665
P. lilacinus	OTU92	China	MN202751
P. lilacinus	UH.1552.175	USA	MZ374592
P. lilacinus	FC18	India	MT672601
P. lilacinus	NKCM1001	Japan	AB244777

MUSCLE algorithm implemented in MEGA X (Tamura et al., 2013). The DNA sample with the minimum Bayesian information criterion (BIC) value is expected to have the best substitution pattern. More than fifty nucleotide sequences were used in this analysis to construct maximum likelihood phylogenetic trees of these studied fungal isolates separately. For Bayesian inference of the phylogenetic reconstruction, the ITS region sequence data were used to construct a phylogenetic tree of every isolate of entomopathogenic fungi (Hung and Posta, 2017).

2.5. Total biomass and blastopore production of *B. bassiana* with different carbon and nitrogen sources

Carbon and nitrogen supply both impact entomopathogens' biomass and blastopore development. The Czapeks Dox broth medium (Difco-Czapek-Dox Broth) was used as a control and was adjusted to examine the effect of different carbon and nitrogen sources at varying concentrations. Carbon sources included sucrose, lactose, starch, and dextrose at 1% and 2% concentrations. Nitrogen sources were $NaNO_3$, NH_4NO_3, KNO_3, and $(NH_4)SO_4$ with 1% and 2% concentrations. After serializing media with the optimized concentration of carbon and nitrogen source, inoculation was done in a laminar flow and then incubated at 28 °C and 90 rpm. After 8–9 days, a sterilized cheesecloth filter weighing 1 g was used to remove mycelium from the media once fungus development was complete. The blastospore-rich medium was then transferred to 15-mL centrifuge tubes and centrifuged at 2000 rcf for 5 min

to extract the blasts. Using Tween 80 0.01% (v/v), the pellet was resuspended and centrifuged again to remove any remaining culture media, and the supernatant was discarded. Blastopore and biomass production were measured. Blastopores were counted using the serial dilution method (propagules/mL), and biomass was determined by filtering the blastopore suspension with a cheesecloth filter and drying it in an oven at 60 °C for 2 days. Total biomass was calculated as follows:

Total biomass = Wt. of oven-dried – Wt. of cheesecloth filter

2.6. Evaluation of food grains for conidial production of the promising strain of entomopathogens

The crushed grains of sorghum (*Sorghum vulgwere*), rice (*Oryza sativa* L.), pearl millet (*Pennisetum typhoides* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and millet (*Pennisetum glaucumi*) were assessed for their suitability as substrates for spore production. After seed crushing, sterile distilled water was added to each substrate to maintain the moisture content at 50%. After thoroughly mixing, the bottles were plugged with cotton and autoclaved. Afterward, each medium was inoculated with a 2 mm diameter mycelial plug obtained from a 14-day-old culture grown on PDA at 28 °C in the dark. The bottles were incubated in the incubator at 28 ± 1 °C. Three replications were maintained for each treatment. Conidia harvesting began on the 10th day after inoculation and continued every 5th day thereafter for up to 25 days, with 1 gram of cultured material sampled each time. Serial dilutions were then prepared on PDA plates using the dilution method.

2.7. Field efficacy of *Beauveria bassiana against H. armigera* on cotton crop

Field experiments were performed to check the efficacy of different conidial concentrations dosage of *B. bassiana* in cotton crop variety "NIAB 78" against *H. armigera* at the Auriga Research Station, Lahore. The experiment was arranged as randomized complete block design with six treatments (T_1, T_2, T_3, T_4, T_5, and T_6) and replicated thrice. T_1 was the untreated control, while T_2 was water only. T_3 to T_5 included *B. bassiana* (Bb04) at conidial concentrations of 2×10^9, 2×10^8, and 2×10^7 (cfu/mL), and T_6 included a chemical insecticide nitenpyram (Table S1). The dead insects on randomly selected ten plants were counted. Data were recorded 1 day before application, on the 3rd day, and on the 7th day after the treatments were applied (Mahr et al., 2001; Mohammed and Hatcher, 2017).

2.8. Statistical analysis

ANOVA was applied to determine the significance level ($p < 0.05$) and means were compared by Duncan's multiple range test. The collected data were subjected to a pooled variance analysis. Wherever needed, percentage standards were converted to arc sin values, whereas root conversion (x + 0.5) was used for larval or nymphal counts (total and mycoses).

3. Results

3.1. The occurrence of entomopathogens in different ecological niches in Punjab

Eight isolates of entomopathogens including *M. anisopliae* (01 No.), *A. lecanii* (01 No.), *P. lilacinus* (02 No.), and *B. bassiana* (04 No.) were isolated. *M. anisopliae* was found on grey weevils in cotton crops with an infection rate ranging from 70% to 80%. *A. lecanii* was also found in moths collected from Lahore. During the survey, multiple strains of *B. bassiana* were found on planthoppers, jassids, grey weevils, and red cotton bugs in cotton and rice in various climatic regions such as Sialkot, Rhim Yar Khan, Multan, Lahore, and Sadiq Abad (Table 1).

3.2. Morphological and cultural characteristics of the fungal isolates

B. bassiana conidiogenous cells were compactly bunched or spiraled or introverted, colorless, short, with a globose base or flask-like base, and increased apically, branching a short distance under each of numerous apically formed conidia. The conidial shape was globose, subglobose, and globose in clusters. The color of the conidia was white to pinkish grey, but it changed from white to pale yellow with time. Conidiophores were colorless, and their heads commonly fused with each other (Fernandes et al., 2006).

Conidiophores of *M. anisopliae* were compact, resembling somatic patches, with phialides bearing conidiogenous cells arranged in whorls, organized in a candle-like appearance. They were cylindrical, and the conidia were single-celled, hyaline, slightly pigmented, and formed long chains (Glare et al., 2021). *A. lecanii* also had erect and verticillate conidiophores with loosely delimited whorls of phialides conidiogenous cells. Phialides were usually awl-shaped with a slightly expanded base. Conidia were single-celled, hyaline organisms with slimy heads and smooth walls (Sugimoto et al., 2003). *P. lilacinus* colonies were spherical and initially white before turning wine-red; the reverse was predominantly dark brown before turning lavender (Dong et al., 2012).

Morphologically, all the isolates showed typical characteristics of *M. anisopliae*, *A. lecanii*, and *P. lilacinus*. Ml 01 isolate from grey weevil showed slight yellowish pigment with green-colored conidia. In the case of *A. lecanii*, Vl01 exhibited pinkish pigmentation with white-colored conidia. The *P. lilacinus* conidiophores are well-developed with septate and hyaline conidiogenous cells. The conidia are produced singly in number with lemon shape and greyish purple color. Conidial heads were frequently compacted with a short conidial chain. The morphological characteristics of all *B. bassiana* isolates

differed in hyphal and conidial characteristics. The isolates differed in terms of conidial color and pigmentation. Bb02 and Bb03 produced greyish conidia, while the other isolates produced white conidia. Bb03 and Bb02 started sporulation in 8–10 days, whereas the remaining isolates began sporulation on PDA media in 7–10 days.

All entomopathogenic fungal isolates (*B. bassiana*, *M. anisopliae*, *V. lecanii*) shared the same microscopic characteristics described in the atlas of entomopathogenic fungi (Figure 1) (Nguyen et al., 2017).

However, diversity was found between the studied strains. We found variations in days for sporulation, colony diameter, conidial yield, and time to cover the specified food for mass production. *A. lecanii* isolated from moths exhibited the lowest conidia yield (3.21×10^9) within the shortest period (8 days) to cover the whole diet. When we employed liquid broth for solid substrate fermentation, *B. bassiana* isolated from grey weevil and red cotton bug from different locations had the highest conidial production (3.3×10^9) and took the longest (8–10 days) to cover the complete diet. *P. lilacinus* strains were isolated from jassids and red cotton bugs in Sialkot and Multan regions. When we employed liquid broth for solid substrate fermentation, the average conidial

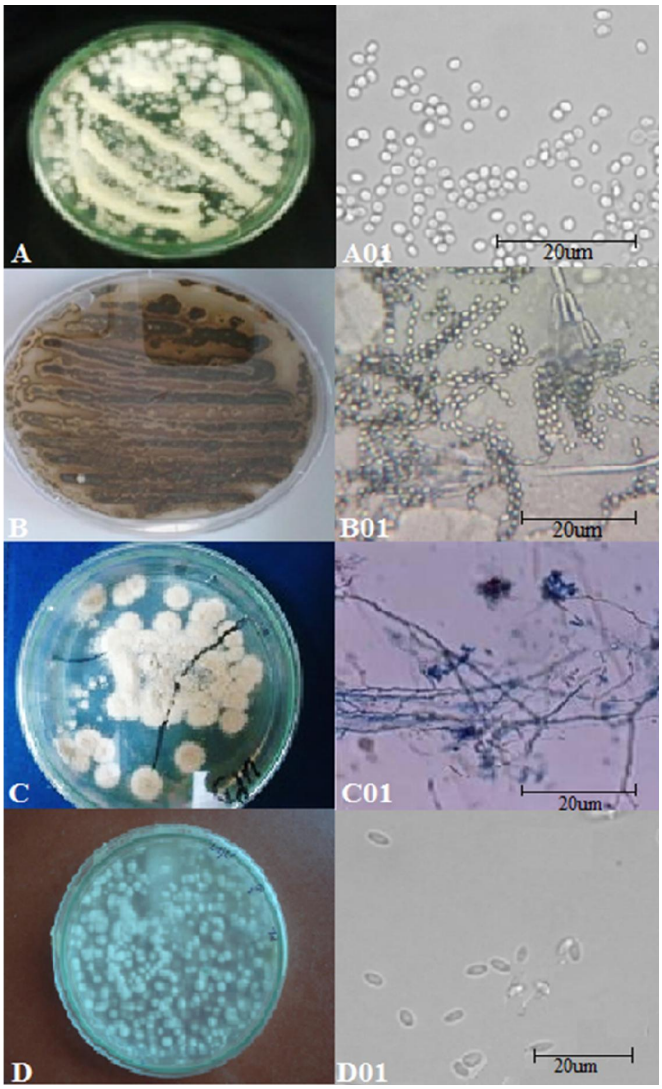

Figure 1. Morphological and cultural patterns of isolated entomopathogenic fungi. A and A01 represent the culture and conidial shape of *Beauveria bassiana*, while B and B01 display the culture and spore l shape of *Metarhizium anisopliae*. C and C01 showcase the culture and conidial shape of *Akanthomyces lecanii*, and D and D01 exhibit the culture and conidial shape of *Paecilomyces lilacinus*.

production was (2.1×10^9), and it took the longest (9–12 days) to cover the whole diet (Table 3, Table S2).

3.3. Pathogenicity of entomopathogenic fungi against H. *armigera*

All the entomopathogenic isolates of *B. bassiana*, *M. anisopliae*, *V. lecanii*, and *P. lilacinus* were evaluated against *H. armigera* and then reared on insect culture plates and supplemented with leaves on 2% agar gel at room temperature. These insect plates were observed continuously, and mortality data were recorded for *H. armigera* after 72 h. The statistical and visual results are presented in Figures 2 and 3, and supplementary material is presented in Table S3. *B. Bassiana* (Bb04) showed a highly significant mortality percentage against *H. armigera*. *B. bassiana* strain Bb01 exhibited the least mortality against the tested insects. *A. lecanii* (Vl01), *M. anisopliae* (Ma01), *P. lilacinus* (Pl01), and (Pl02) showed higher mortality percentages but required more than 72 h to achieve mortality. Based on these results, the strain of *B. Bassiana* (Bb04) was selected for further studies.

3.4. Molecular identification

The ITS region of all studied entomopathogenic fungal isolates was successfully amplified with different ITS primer pairs. BLAST analysis of the sequences obtained revealed high similarity (99% to 100%) between the ITS regions of the studied isolates and identified four isolates as *B. bassiana*, one isolate as *M. anisopliae*, one isolate as *A. lecanii*, and two isolates as *P. lilacinus.* The obtained sequences were deposited in GenBank, and accession numbers are shown in Table 4.

3.5. DNA modeling and phylogenetic analysis

DNA models were examined based on their Akaike information criterion (AIC), BIC, InL, and nucleotide frequencies. The GTR model was determined to be the best model for phylogenetic analysis of the DNA sequence of the *B. bassiana* ITS region because it attained the lowest BIC value of 46,602.95 and exhibited the best substitution pattern. The JC model was determined to be the best for phylogenetic analysis of the DNA sequence of the *M. anisopliae*, *A. lecanii*, and *P. lilacinus* ITS region because it attained the lowest BIC value (2162.66, 1916.75, and 1916.75, respectively) with the best substitution pattern. The phylogenetic consensus trees of the studied entomopathogenic fungal isolates were developed separately based on Bayesian inference and the bootstrap technique. Three maximum-likelihood trees were constructed using the sequences of the ITS region of *B. bassiana* (Bb01, Bb02, Bb03, Bb04), *M. anisopliae* (Ma01), *A. lecanii* (Vl01), and *P. lilacinus* (Pl01, Pl02) isolates from a different region of Pakistan and ITS sequences of these studied isolates that were obtained from GenBank separately (Figures 4–7). Phylogenetic analysis based on the ITS region of different entomopathogenic fungi and their strains and reference strains of various species of entomopathogenic fungi by the maximum-likelihood method with Tamura's three-parameter model using the MEGA X software program confirmed the identification of fungi.

3.6. Effect of nitrogen and carbon sources on the growth and sporulation of B. *bassiana* (Bb04)

B. bassiana (Bb04) growth was affected by the different sources and concentrations of carbon and nitrogen in

Table 3. Morphological and cultural characters of entomopathogenic fungal isolates.

Strain	Conidia				Colony (mm) [W]	Sporulation [Z]	Plate cover [T]	Sporulation (days) [Y]
	Color	Shape	Length (µm)	Width (µm)				
Bb01	White	Globose	3.4bcd	2.6c	38.6d	2.3a	11b	7a
Bb02	grayish	Globose	3.9abcd	3.1a	32.30e	1.53a	14a	8a
Bb03	Grayish	Globose	3.8abcd	2.7bc	33.19e	1.62a	14a	8a
Bb04	White	Subglobose	4.1ab	2.9ab	41.1c	1.34a	10bc	7a
Ma01	Green	Ellipsoid	6.5a	2.2d	32.30e	1.53a	10bc	8a
Vl01	White	Oval to oblong	5.9abc	2.2d	52.13a	1.41a	8d	5b
Pl01	White with grayish shade	Fusiform	2.1cd	2.8bc	43.23b	1.2a	9cd	8a
Pl02	White with pinkish shade	Ellipsoid	1.8d	2.3d	4.11f	1.34a	10bc	7a

The morphological and growth properties of the Entomopathogenic fungal isolates are used to organize them. At the 0.05 level, the mean difference between the two groups is significant; based on Tukey's test, values with the same letters do not differ significantly within a column.

[W] On potato dextrose agar, growth rate (colony diameter) was monitored daily from the 5th to the 20th day following inoculation (mm day^{-1}).

[Z] Sporulation of different fungal isolates per plate ($\times 10^9$ cfu) on the 10th day.

[T] Number of days required for studied fungal isolates to cover the whole Petri plate.

[Y] Number of days required for studied fungal isolates to begin sporulation.

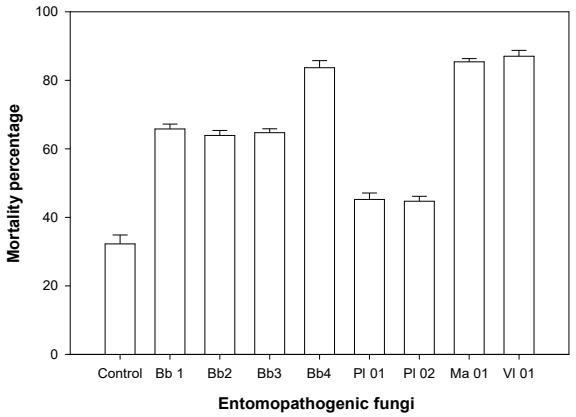

Figure 2. Mortality percentage of *H. armigera* with a different strain of entomopathogenic fungi after 72 h of blastopore application. The X-axis represents the different entomopathogenic fungi and their strains. In the x-axis, Bb1, Bb2, Bb3, and Bb4 represent the different strains of *Beauveria bassiana*, and Pl01 and Pl02 represent the different strains of *Purpureocillium lilacinus*. Ma01 represents the strain of *M. anisopliae*, Vl01 represents the strain of *Akanthomyces lecanii*, and Y-axis represents the mortality percentage of *H. armigera* ($p < 0.001$).

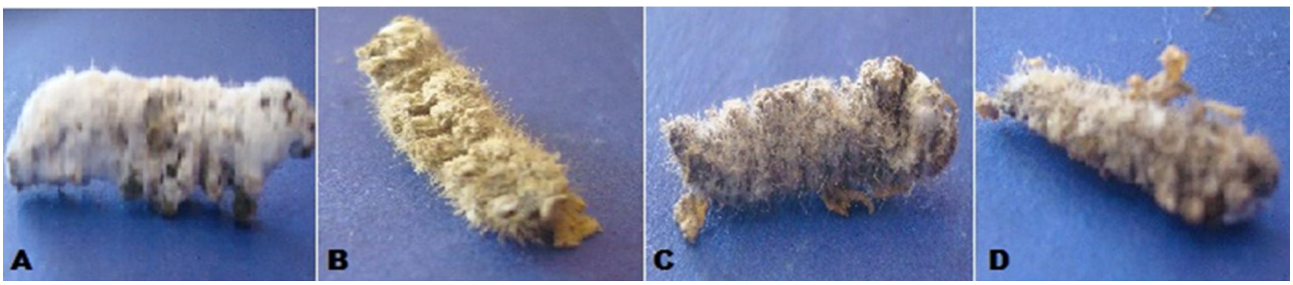

Figure 3. Entomopathogenic fungi *Beauveria bassiana* (A), *Metarhizium anisopliae* (B), *Akanthomyces lecanii* (C), and *Purpureocillium lilacinus* (D) colonized on *H. armigera.*

Table 4. Deposited sequences of entomopathogenic fungal studied Strains in the NCBI GenBank database.

Fungi	Strain	Isolation source	ITS deposited sequences accession no.
Beauveria bassiana	Bb01	Grey weevil	OL825007
	Bb02	Grey weevil	OL825008
	Bb03	Red cotton bug	OL825009
	Bb04	Red cotton bug	OL825010
Metarhizium anisopliae	Ma01	Grey weevil	OL839175
Lecanicillium lecanii	Vl01	Red cotton bug	OL827536
Paecilomyces lilacinus	Pl01	Jassid	OL82756
	Pl02	Moth	L827569

Czapeck-Dox broth. The results showed that the total dry mycelia weight was significantly higher than that of control for 1% starch concentrated medium followed by 1% sucrose, 1% dextrose, and 1% lactose, respectively. Blastopore formation was highest in 1% starch concentrated medium, followed by 1% sucrose and 1% dextrose. One percent lactose showed the lowest blastopore production. These data were recorded on the 8th day after inoculation. With a 2% concentration of carbon sources, dextrose showed high growth, followed by sucrose and lactose,

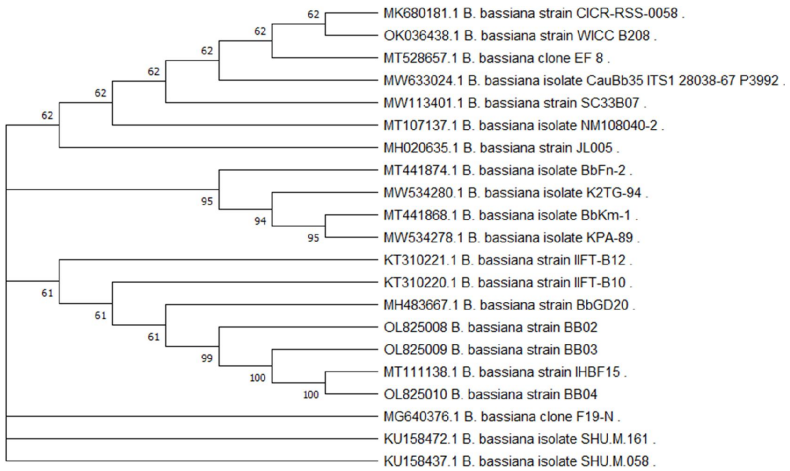

Figure 4. Maximum-likelihood phylogram inferred from partial ITS sequence data showing the phylogenetic relationship between *Beauveria bassiana* (Bb01, Bb02, Bb03, Bb04) strains isolated from planthoppers, grey weevils, red cotton bugs, and jassids in Pakistan (Bb01 to Bb04) and all the available *Beauveria* species sequences obtained from GenBank. The genus and species names of the GenBank sequences are shown. The numbers on the node of branches indicate the phylogenetic relationship among the isolates in terms of branch length. The tree with the highest log-likelihood value is –781.57.

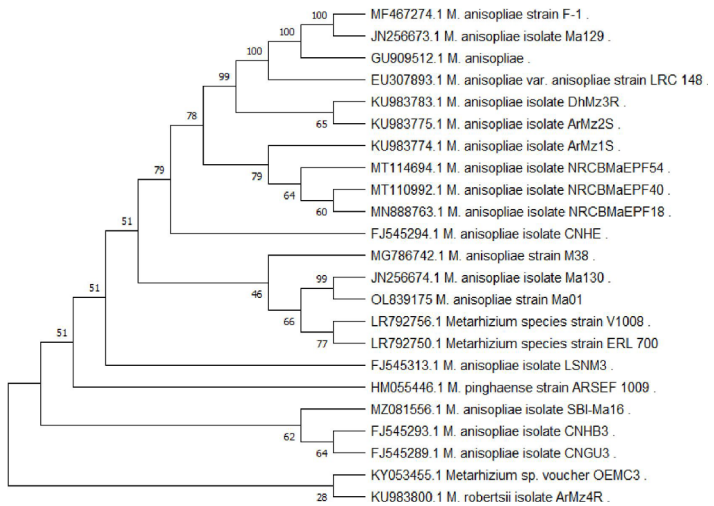

Figure 5. Maximum-likelihood phylogram inferred from partial ITS sequence data showing the phylogenetic relationship between *Metarhizium anisopliae* (Ma01) strains isolated from grey weevils in Pakistan. *Metarhizium anisopliae* (Ma01) and all the available *Metarhizium* species sequences were obtained from GenBank. The genus and species names of the GenBank sequences are shown. The numbers above the branches indicate the phylogenetic relationship among the isolates regarding branch length. The tree with the highest log-likelihood value is –876.35.

respectively. Starch showed almost negligible fungal growth as indicated by dry mycelial weight. Blastopore formation at a 2% concentration of carbon source "starch" exhibited the highest blastopore production. However, the values did not significantly differ from each other, indicating nonsignificant results. Further numerical results are given in Figure 8 and Table S4. Nitrogen sources were $NaNO_3$, NH_4NO_3, KNO_3, and $(NH_4)SO_4$ with 1% and 2% concentrations. The results indicated that the total dry weight of mycelia in the 1% $NaNO_3$ and 1% NH_4NO_3

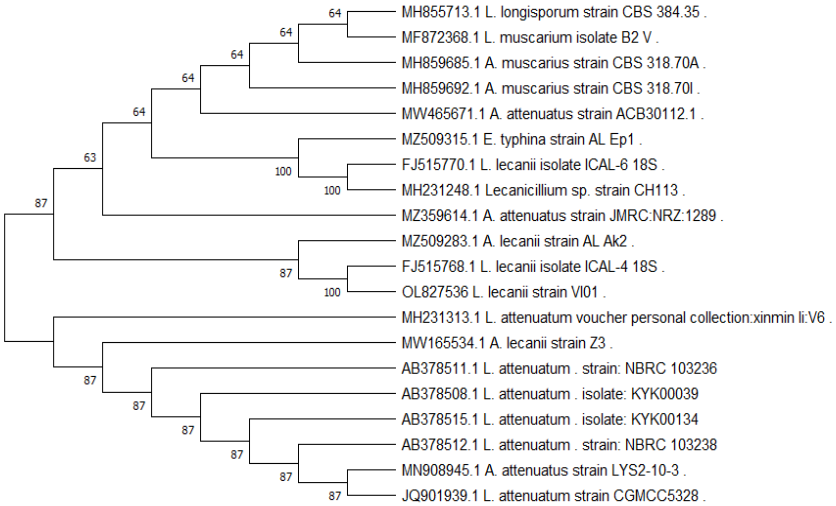

Figure 6. Maximum-likelihood phylogram inferred from partial ITS sequence data showing the phylogenetic relationship between *Akanthomyces lecanii* (Vl01) strains isolated from the moths in Pakistan. *Akanthomyces lecanii* (Vl01) and all the available *Lecanicillium* species sequences were obtained from GenBank. The genus and species names of the GenBank sequences are shown. The numbers on the node of the branches indicate the phylogenetic relationship among the isolates in terms of branch length. The tree with the highest log-likelihood value is –786.29.

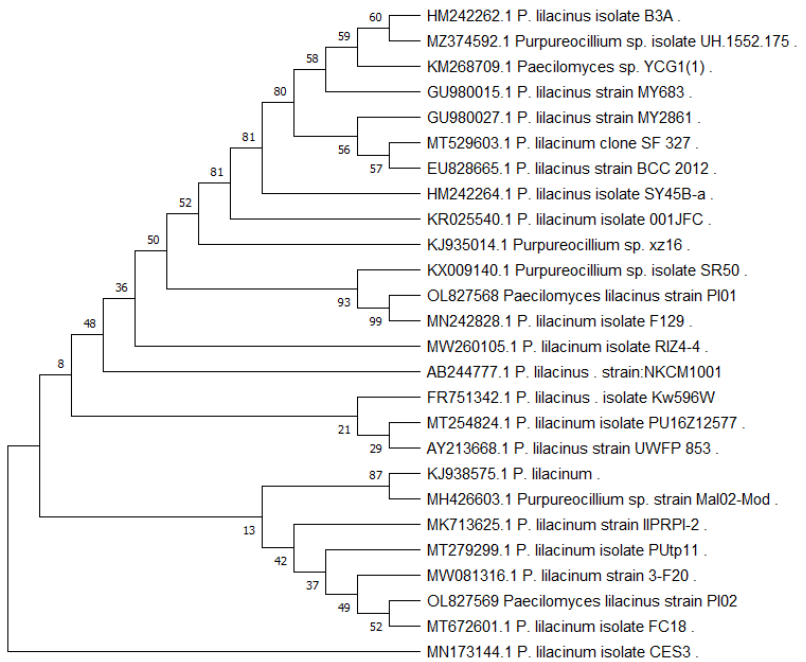

Figure 7. Maximum-likelihood phylogram inferred from partial ITS sequence data showing the phylogenetic relationship between *Purpureocillium lilacinus* (Pl01) strain isolated from spotted bollworms and Pl02 strain isolated from grey weevils in Pakistan. *Purpureocillium lilacinus* (Pl01, Pl02) and all the available *Paecilomyces* species sequences were obtained from GenBank. The genus and species names of the GenBank sequences are shown. The numbers on the node of the branches indicate the phylogenetic relationship among the isolates in terms of branch length. The tree with the highest log-likelihood value is –1113.66.

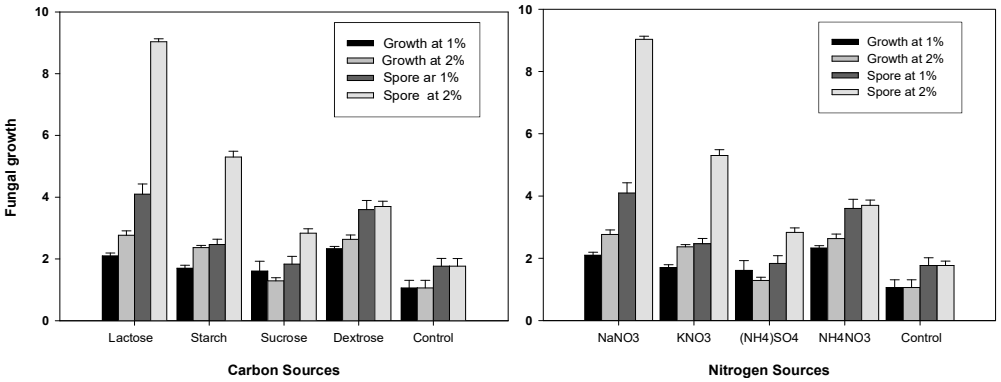

Figure 8. Effect on fungal growth of *B. bassiana* (Bb04) using different carbon and nitrogen sources and their different concentrations ($p < 0.001$).

concentrated medium was not significant, but significant differences were observed with 1% KNO_3 and 1% $(NH_4)SO_4$. The 2% concentration of NH_4NO_3, KNO_3, and $NaNO_3$ did not yield significant results, but they exhibited a significant difference when compared to the medium containing $(NH_4)SO_4$. Blastopore formation is also affected by concentration variation. The highest blastopore formation was observed in the medium with 2% NaNO3, followed by 2% KNO_3 in second place, and 2% NH_4NO_3 in third place. $(NH_4)SO_4$ exhibited the lowest blastopore production. Statistically, there were no significant differences observed among all nitrogen sources at both concentrations. These data were recorded on the 8th day after inoculation. Further numerical results were given in Figure 8 and Table S5.

3.7. Evaluation of food grains for conidial production of a promising strain of entomopathogens

Food grains were also used for the mass production of different fungal strains, so different grains were used to evaluate the conidial production of *B. bassiana* (Bb04). For this purpose, we used sorghum, rice, maize, millet, wheat, and pearl millet crushed grains. The study focused on the time required for mycelia startup, conidial formation, and the number of conidia on the 20th day following inoculation. The mycelial mat and sporulation increased day by day after the inoculation. On the 20th day, mycelium covered the entire grain surface. The sporulation varied with varying grain sources at different intervals (Figure 9). Maize proved to be the best substrate for the growth of *B. Bassiana* and showed a significant conidial yield (6.8×10^9 cfu/mL). Sorghum followed maize grain with a conidial yield of 3.3×10^9 (cfu/mL). Rice and millet substrates showed similar effectiveness in supporting conidial development. Wheat turned out to be the poorest substrate for culturing *B. bassiana*.

3.8. Field efficacy of B. *bassiana* (Bb04) against H. *armigera*

For in vitro control of *H. armigera*, we used foliar sprays with varied concentrations of *B. bassiana* (Bb04) on the cotton field. This experiment employed three foliar applications, and insect population data was collected both before and 3 and 4 days after the foliar application of *B. bassiana.* The collected data revealed that treatments "T3," "T4," and "T5," based on *B. bassiana* (Bb04) pesticide, did not show significant differences on the third day after application (DAA). However, a difference in mortality percentage was observed on the 7th day after application, with the mortality curve reaching its peak following the second and third applications. The nitenpyram-based treatment resulted in the highest mortality on the 3rd day after application (DAA). Nonetheless, by the 7th day after the first application, the number of living *H. armigera* had increased. The *B. bassiana* (Bb04)-based pesticide treatment "T_5" (2×10^7) showed mortality after the third foliar application. After the 1st application, all bio pesticide-based treatments had no significant difference on the 3rd day after the first application (DAA). This trend changed on the 7th day after the first application. We observed that with higher spore concentration treatments, the mortality rate increased, and the mortality percentage curves rose over time. This trend was not observed in the chemical control treatment "T_6" which showed maximum mortality percentage on the day of foliar application, but living *H. armigera* percentage became higher over time. Statistical analysis of these three foliar applications showed that the first application treatments, "T_1", "T_2", "T_4", and "T_5", were not significantly different from each other. However, the treatments "T3" and "T6" exhibited slightly nonsignificant results compared to treatments "T4" and "T5." After the second spray, nonsignificant results began to appear, with treatments "T1" and "T2" becoming nonsignificant to each other. Significant results were still observed between the other treatments. Treatments "T3," "T4," and "T5" were nonsignificant to each other but showed significant differences when compared to the other treatments. "T_6" treatment showed a significant result among all treatments. During the observation of the 3rd foliar application, the first two treatments were not significantly different from

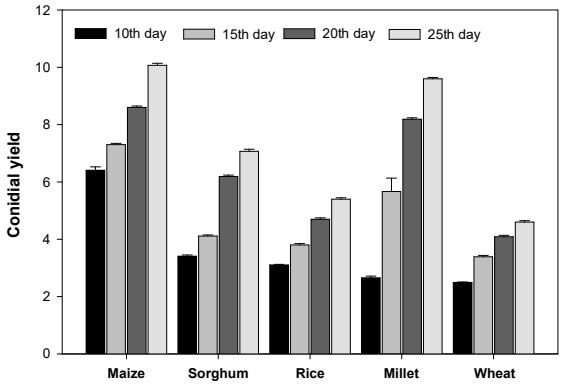

Figure 9. Conidial production of *B. bassiana* (Bb04) using different seed-based media on different days ($p < 0.001$).

each other. Treatment "T5" exhibited a significant result among all treatments, while "T4" was not significantly different from treatments "T5," "T3," and "T6," but showed significance for treatments "T1" and "T2." These results are shown in Figures 10 and 11 and Tables S6 and S7.

4. Discussion

The ecology and method of entomopathogenic fungi hold great promise for application in sustainable agriculture (Becher et al., 2018). The current study aimed to identify the most effective entomopathogenic fungi for the biological control of various agricultural insects. Various entomopathogenic fungal strains (*M. anisopliae*, *A. lecanii*, *B. bassiana*, *P. lilacinus*) were isolated from different districts of Punjab for this purpose (Wakil et al., 2013). The disease incidence frequency may alter as the agroclimatic region changes (Lydia et al., 2017). The infection frequency of the investigated entomopathogens was high in northern and southern Punjab (Waheed et al., 2012). The northern area had a virtual humidity of 80%–89% and a temperature of 26.5–27.7 °C, whereas southern Punjab had a lower humidity of 75%–78% and a higher temperature of 30–32.5 °C. We may conclude that the potential use of *B. bassiana* as a biopesticide is dependent on environmental conditions.

A total of eight entomopathogenic fungal strains were isolated, cultured, and identified on a morphological, cultural, and molecular basis (Khan et al., 2016; Hung and Posta, 2017; Teja and Rehamn, 2017). Out of the eight fungal strains, four were identified as *Beauveria bassiana* (Bb01, Bb02, Bb03, and Bb04), two as *Purpureocillium lilacinus* (Pl01, Pl02), one as *Metarhizium anisopliae* (Ml01), and one as *A. lecanii* (Vl01).

The pathogenicity of different isolates of entomopathogenic fungi was assessed, revealing varied insecticidal activity among all fungi. Additionally, differences in insecticidal effectiveness were observed among different strains of a single entomopathogenic fungus (Nguyen et al., 2017). The *B. bassiana* strain Bb04 showed a significant mortality percentage against fall armyworms. *M. anisopliae* (Ma01) exhibited the highest mortality percentage against moths but not against fall armyworms, and it yielded minimal results against spotted worms (Teja and Rehamn, 2017). Based on these results, *B. bassiana* (Bb04) strain was selected for further studies. The nutrients play a fundamental role in fastening fungal growth and sporulation (Kichaoui et al., 2017). Carbon and nitrogen have a fundamental role in the growth and sporulation of entomopathogenic fungi (Bugti et al., 2018). Therefore, the effect of different carbon and nitrogen sources with different concentrations on the growth and sporulation of *B. bassiana* was analyzed in the Czapeck-Dox broth medium. With a 2% sucrose concentrated medium, the total dry mycelia weight was much higher.

The production of blastopore was most significant in a solution containing 2% starch. In the case of 1% carbon sources, sucrose showed much higher growth and blastopore formation, but their values could not cross the significant range; therefore, the obtained findings were nonsignificantly different from each other Figure 8 (Singh et al., 1972). For fungal growth, nitrogen enlarges the hyphae and extends the fungal occupying substrate (Mehta et al., 2012). Therefore, specific nitrogen resources were applied with different concentrations to check their effect on *B. bassiana* (Bb04). The findings revealed that the total dry mycelial weight of *B. bassiana* (Bb04) was notably higher in a 1% NH_4NO_3 concentrated medium compared to other media. In the case of a 2% concentration of NH_4NO_3, KNO_3, and $NaNO_3$, no significant difference was observed among them. However, a substantial difference was noted with the medium containing $(NH_4)SO_4$. Concentration changes had an effect on blastopore production as well. Figure 9 (Dhar and Kaur, 2010) shows that 2% $NaNO_3$ produced the most blastopore compared to other media.

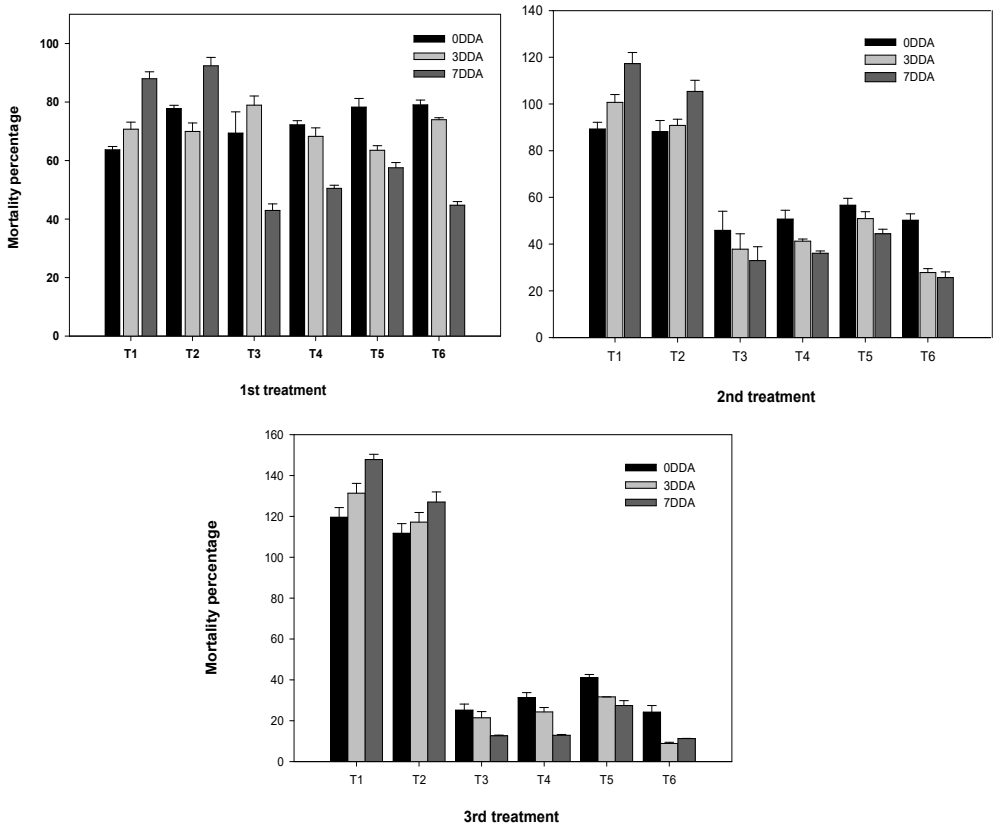

Figure 10. Comparative analysis of the foliar applications of different conidial concentrations of *B. bassiana* (Bb04) and biopesticide efficacies compared with chemical control against *H. armigera*. Section "A" represents the results of the first applied treatment, Section "B" represents the results of the second applied treatment, and Section "C" represents the results of the third applied treatment. The Y-axis represents the percentage of mortality of *H. armigera* resulting from different treatments. The X-axis represents the different treatments applied against *H. armigera*. "T1" was considered a control treatment, so no treatment was applied in the "T1" block. In "T2" treatment, water was applied, in "T3" treatment *B. bassiana* (Bb04) conidial concentration was 2×10^9 cfu/mL, in "T4" treatment, *B. bassiana* (Bb04) conidial concentration of fungus was 2×10^8 cfu/mL, in "T5" treatment, *B. bassiana* (Bb04) conidial concentration of fungus was 2×10^7 cfu/mL, and in "T6" treatment, chemical insecticide nitenpyram was applied to the cotton crop for controlling *H. armigera*.
The p-value of the first applied treatment was <0.63 on the 1st day, <0.52 on the 2nd day, and <0.002 on the 3rd day. The p-value of the second applied treatment was <0.00 on the 1st, 2nd, and 3rd days. The p-value of the third applied treatment was <0.00 on the 1st and 3rd days, and <0.0029 on the 2nd day.

Comparison of strains revealed that *B. bassiana* (Bb04) has great potential to effectively manage insect pests when adequately developed as a biopesticide (Ibrahim, 2012). Therefore, the in vitro experiment was performed to check the efficacy of *B. bassiana* (Bb04) against *H. armigera* compared to chemical pesticides. The biopesticide did not yield any significant results, but T_6 (nitenpyram) treatment notably achieved the highest peak of mortality on the 3rd day after the 1st application (DAA). The biopesticide effects were observed on the 7th DAA. We noticed that in T_6, the number of *H. armigera* rose again, but in T_3, T_4, and T_5, the number of *H. armigera* gradually decreased. On the 7th day after the third application, we observed that T_3, T_4, T_5, and T_6 treatments were not significantly different from each other, and the biopesticide gradually approached the mortality rate of the chemical pesticide (Prasad and Pal, 2014). The lower mortality of the fungus compared to chemicals may be attributed to the fact that chemical insecticides act quickly, leading to a significant reduction in the whitefly population. In contrast, entomopathogens act more gradually on the pests, allowing them to survive on the plant while waiting for the disease's incubation time to elapse.

5. Conclusions

We selected *B. bassiana* (Bb04) based on its growth performance on different sources and pathogenicity as

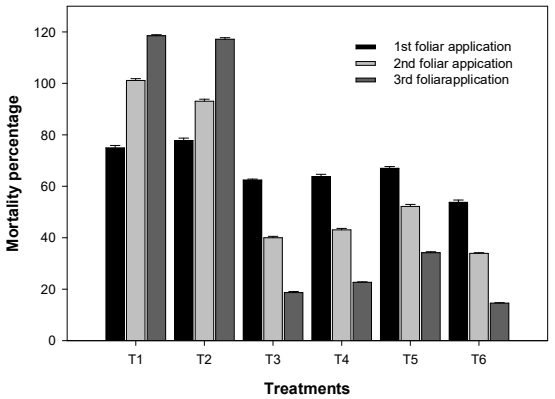

Figure 11. Comparative analysis of all the foliar applications of different conidial concentrations of *Beauveria bassiana* (Bb04) and biopesticide efficacies compared with chemical control against *Helicoverp*a *armigera*. (The comparative p-values for all applications were <0.00 on the 1st, 2nd, and 3rd days.)

the most promising entomopathogenic fungal strain after screening the best entomopathogenic fungi. The total dry weight of mycelia was significantly higher than that of the control for 1% starch concentrated medium followed by 1% sucrose, 1% dextrose, and 1lactose, respectively. The plate pathogenicity assay revealed that *B. bassiana* (Bb04) inflicted the highest mortality to *H. armigera* as compared to other strains. BLAST analysis revealed high similarity (99%–100%) between the ITS regions of the studied isolates. The results confirmed that the use of *B. bassiana* (Bb04) was an eco-friendly and sustainable approach that could replace chemical insecticides.

Contribution of authors

Hafiz Husnain Nawaz designed the study. Hafiz Husnain Nawaz, Javed Iqbal, and Attiq ur Rehman performed the experiments and analysis. Mubashir Hussain and Abid Farid wrote the original draft. Shahzad Toufeeq, Rashid Azad, and Abdul Qayyum provided guidance throughout the study. Javed Iqbal supervised the entire research. Gülşah Keklik, Yamin Bibi, and Khalid Ali Khan provided technical expertise to improve the article and assisted in acquiring funding. All authors reviewed and edited the manuscript.

Acknowledgments

The authors extend their appreciation to University Higher Education Fund for funding this research work under Research Support Program for Central labs at King Khalid University through the project number CL/PRI/A/4.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Data availability statement

All the data related to this study is presented in the main text.

References

Alford, DV (2000). Biological control of insect pests on oilseed rape in Europe. The Royal Society of Chemistry. 11: 200-202. https://doi.org/10.1039/b008023n

Ashraf M, Farooq M, Shakeel M, Din N, Hussain S et al. (2017). Influence of entomopathogenic fungus, Metarhizium anisopliae, alone and in combination with diatomaceous earth and thiamethoxam on mortality, progeny production, mycosis, and sporulation of the stored grain insect pests. Environmental Science and Pollution Research. 24: 28165-28174. https://doi.org/10.1007/s11356-017-0383-6

Becher PG, Jensen RE, Natsopoulou ME, Verschut V, De Fine Licht HH (2018). Infection of Drosophila suzukii with the obligate insect-pathogenic fungus Entomophthora muscae. Journal of Pest Science. 91: 781-781. https://doi.org/10.1007/s10340-017-0915-3

Bugti G.A, Bin W, Na C, Feng L.H (2018). Pathogenicity of Beauveria bassiana strain 202 against sap-sucking insect pests. Plant Protection Science. 54 (2): 111-117. https://doi.org/10.17221/45/2017-PPS

Dhar P, Kaur G (2010). Effects of carbon and nitrogen sources on the induction and repression of chitinase enzyme from Beauveria bassiana isolates. African Journal of Biotechnololgy. 9 (47): 8092–8099. https://doi.org/10.5897/ajb09.1161

Dong Q, Wang H, Xing X, Ji S (2012). Identification and characterization of a special species of Paecilomyces. Annals of Microbiology. 62: 1587-1592. https://doi.org/10.1007/s13213-011-0414-3

Faria MR, D. Wraight SP (2007). Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. Biological Control. 43 (3): 237-256. https://doi.org/10.1016/j.biocontrol.2007.08.001

Fernandes EKK, Costa GL, Moraes ÁML, Zahner V, Pinheiro Bittencourt VRE (2006). Study on morphology, pathogenicity, and genetic variability of Beauveria bassiana isolates obtained from Boophilus microplus tick. Parasitology Research. 98: 324-332. https://doi.org/10.1007/s00436-005-0058-y

Ferrigo D, Causin R, Raiola A (2017). Effect of potential biocontrol agents selected among grapevine endophytes and commercial products on crown gall disease. BioControl. 62: 821-833 https://doi.org/10.1007/s10526-017-9847-3

Glare TR, Scholte Op Reimer Y, Cummings N, Rivas-Franco F, Nelson TL, Zimmermann G (2021). Diversity of the insect pathogenic fungi in the genus Metarhizium in New Zealand. New Zealand Journal of Botany. 59: 440-456. https://doi.org/10.1080/0028825X.2021.1890155

Hoddle M, Van Driesche, R (2009). Biological control of insect pests. In: Encyclopedia of Insects. Amsterdam, the Netherlands, Elsevier, pp. 91-101.

Hung DV, Posta K (2017). PCR based detection of entomopathogenic fungus Metarhizium anisopliae in host organisms. ARPN Journal of Agricultural and Biological Science. 12 (2): 38-42.

Husnain H, Shahid AA, Ul-Haq MI, Ali A, Muhammed U et al. (2014). Efficacy of entomopathogenic fungi as biological control agent against insect pests of Gossypium hirsutum. Journal of Natural Sciences Research. 4 (5): 68-72.

Hussain M, Ghazanfar M, Malik MF, Umar M, Younas, M (2021). Effect of endectocides and antibiotic dung poisoning on mortality of dung beetle species: Antibiotic dung poisoning in diferent species. Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences. 64 (3): 211-216.

Ibrahim D (2012) Characterization of solid state fermentation culture conditions for growth and mananase production by Aspergillus niger usm f4 on rice husk in tray system. Biotechnology Journal International. 2 (3): 133-145. https://doi.org/10.9734/bbj/2012/1486

Khan S, Nadir S, Lihua G, Xu J, Holmes KA et al. (2016). Identification and characterization of an insect toxin protein, Bb70p, from the entomopathogenic fungus, Beauveria bassiana, using Galleria mellonella as a model system. Journal of Invertebrate Pathology. 133: 87-94. https://doi.org/10.1016/j.jip.2015.11.010

Kichaoui AY, El Asaker BAA, El-Hindi MW (2017). Isolation, molecular identification and under Lab evaluation of the Entomopathogenic Fungi M. anisopliae and B. bassiana against the Red Palm Weevil R. ferrugineus in Gaza Strip. Advances in Microbiology. 7: 109-124. https://doi.org/10.4236/aim.2017.71009

Kin PK, Azmi WA, Kamarudin N, Ali SRA, Moslim R (2017.) The occurrence of entomopathogenic fungi on mineral and peat soils in peninsular Malaysia. American Journal of Agricultural and Biological Sciences. 12: 1-12. https://doi.org/10.3844/ajabssp.2017.1.12

Komaki A, Kordali Ş, Usanmaz Bozhuyuk A, Altinok HH, Kesdek M et al. (2017). Laboratory assessment for biological control of Tribolium confusum du Val., 1863 (Coleoptera: Tenebrionidae) by entomopathogenic fungi. Turkish Journal of Entomology. 41 (1): 95-103. https://doi.org/10.16970/ted.80578

Kryukov VY, Tomilova OG, Luzina OA, Yaroslavtseva ON, Akhanaev YB et al. (2018). Effects of fluorine-containing usnic acid and fungus Beauveria bassiana on the survival and immune–physiological reactions of Colorado potato beetle larvae. Pest Management Science 74 (3): 598-606. https://doi.org/10.1002/ps.4741

Kumar S, Nehra M, Dilbaghi N, Marrazza G, Hassan AA et al. (2019). Nano-based smart pesticide formulations: Emerging opportunities for agriculture. Journal of Controlled Release. 294: 131-153. https://doi.org/10.1016/j.jconrel.2018.12.012

Labbé R, Gillespie D, Cloutier C, Brodeur J (2009). Compatibility of an entomopathogenic fungus with a predator and a parasitoid in the biological control of greenhouse whitefly. Biocontrol Science and Technology, 19 (4): 429-446.

Litwin A, Nowak M, Różalska S (2020). Entomopathogenic fungi: unconventional applications. Reviews in Environmental Science and Biotechnology. 19: 23-42. https://doi.org/10.1007/s11157-020-09525-1

Lovett B, St. Leger, RJ (2018). Genetically engineering better fungal biopesticides. Pest Management Science, 74 (4): 781-789. https://doi.org/10.1002/ps.4734

Lydia CH, Sunil V, Renuka K, Sandhya V, Bentur JS (2017). Entomopathogenic fungi as potential bio-control agents and mode of action against insect pests. Agriculture Update. 12: 277-283. https://doi.org/10.15740/HAS/AU/12.TECHSEAR(1)2017/277-283

Mahr SER, Cloyd R, Mahr DL, Sadof CS (2001). Biological control of insects and other pests of greenhouse crops. North Central Regional Publication. 581: 100-108.

Mannino MC, Huarte-Bonnet C, Davyt-Colo B, Pedrini N (2019). Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. Journal of Fungi. 5 (2): 33 https://doi.org/10.3390/jof5020033

Mantzoukas S, Eliopoulos PA (2020). Endophytic entomopathogenic fungi: a valuable biological control tool against plant pests. Applied Science. 10 (1): 360. https://doi.org/10.3390/app10010360

Mehta J, Kaushal N, Sen P, Sharma DR, Dhillon MS (2012). Impact of carbon & nitrogen sources on the Verticillium lecanii and Metarhizium anisopliae - Entomopathogenic fungi. European Journal of Experimental Biology. 2 (4): 1278-1283.

Méndez-González F, Castillo-Minjarez JM, Loera O, Favela-Torres E (2022). Current developments in the resistance, quality, and production of entomopathogenic fungi. World Journal of Microbiology and Biotechnology. 38 (7) 115. https://doi.org/10.1007/s11274-022-03301-9

Mohammed AA, Hatcher PE (2017) Combining entomopathogenic fungi and parasitoids to control the green peach aphid Myzus persicae. Biological Control. 110: 44-55. https://doi.org/10.1016/j.biocontrol.2017.03.012

Nawaz HH, Anam U, Nelly Rajaofera MJ, He Q, Liu W et al (2018). Development of SNP-based markers to identify Colletotrichum gossypii in Upland cotton. Plant Disease. 102: 1426-1433. https://doi.org/10.1094/PDIS-10-17-1672-RE

Nguyen HC, Tran TVA, Nguyen QL, Nguyen NN, Nguyen MK et al. (2017). Newly isolated Paecilomyces lilacinus and Paecilomyces javanicus as novel biocontrol agents for Plutella xylostella and Spodoptera litura. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 45: 280-286. https://doi.org/10.15835/nbha45110726

Ownley BH, Gwinn KD, Vega FE (2010). Endophytic fungal entomopathogens with activity against plant pathogens: Ecology and evolution. BioControl. 55: 113-128. https://doi.org/10.1007/s10526-009-9241-x

Pérez-González O, Sánchez-Peña SR (2017). Compatibility in vitro and in vivo of the entomopathogenic fungi Beauveria bassiana and Hirsutella citriformis with selected insecticides. Southwestern Entomologist. 42 (3): 707-718. https://doi.org/10.3958/059.042.0309

Prasad CS, Pal R (2014). Mass production and economics of entomopathogenic fungus, Beauveria bassiana, Metarhizium anisopliae and Verticillium lecanii on agricultural and industrial waste. Scholars Journal of Agriculture and Veterinary Sciences. 1: 28-32.

Rusch A, Valantin-Morison M, Sarthou JP, Roger-Estrade J (2010). Biological control of insect pests in agroecosystems. Advances in Agronomy. 109: 219-259. https://doi.org/10.1016/B978-0-12-385040-9.00006-2

Sabbahi R, Lavallée R, Merzouki A, Guertin C (2017). Differentiation of entomopathogenic fungus Beauveria bassiana (Ascomycetes : Hypocreales) isolates by PCR-RFLP. Phytoprotection. 90 (2): 49-56. https://doi.org/10.7202/044022ar

Saldarriaga Ausique JJ, D'Alessandro CP, Conceschi MR, Mascarin GM, Delalibera Júnior I (2017). Efficacy of entomopathogenic fungi against adult Diaphorina citri from laboratory to field applications. Journal of Pest Science. 90: 947-960. https://doi.org/10.1007/s10340-017-0846-z

Sani I, Ismail SI, Abdullah S, Jalinas J, Jamian S et al. (2020). A review of the biology and control of whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae), with special reference to biological control using entomopathogenic fungi. Insects. 11: 1-18. https://doi.org/10.3390/insects11090619

Singh SB, Bais BS, Singh DR (1972). Effect of different carbon and nitrogen sources on the growth and sporulation of Claviceps microcephala. Mycopathologia et Mycologia Applicata. 46: 373-378. https://doi.org/10.1007/BF02052134

Sugimoto M, Koike M, Hiyama N, Nagao H (2003). Genetic, morphological, and virulence characterization of the entomopathogenic fungus Verticillium lecanii. Journal of Invertebrate Pathology. 82: 176-187. https://doi.org/10.1016/S0022-2011(03)00014-4

Sugimoto M, Koike M, Nagao H (2001). Ribotyping of entomopathogenic Verticillium lecanii in Japan. Phytoparasitica. 29: 413-420. https://doi.org/10.1007/BF02981860

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 30: 2725-2729. https://doi.org/10.1093/molbev/mst197

Teja KNPC, Rehamn SJ (2017). Evaluation of the growth and sporulation of different entomopathogenic fungi in different liquid and solid media at varied concentrations. International Journal of Bioassays 6: 5459. https://doi.org/10.21746/ijbio.2017.08.003

Ugine TA, Wraight SP, Sanderson JP (2013). Microbial biological control potential of three strains of Beauveria bassiana s. l. against greenhouse shore fly Scatella tenuicosta: Assessment of virulence, mass production capacity, and effects on shore fly reproduction. Biological Control. 65 (3): 348-356. https://doi.org/10.1016/j.biocontrol.2013.03.014

Umar M, Hussain M (2023). Faunistic analysis of insects of Deva Vatala National Park and agroecosystem of Gujrat Pakistan. Kuwait Journal of Science. 50 (1A): 1-25. https://doi.org/10.48129/kjs.15801

Waheed A, Salik NK, Muhammad A, Muhammad SH (2012). Occurrence of insect associated fungi in hot arid zone, Pakist gan. African Journal of Microbiology Research. 6 (44): 6878-6883. https://doi.org/10.5897/AJMR11.774

Wakil W, Ghazanfar MU, Riasat T, Kwon YJ, Qayyum MA et al. (2013). Occurrence and diversity of entomopathogenic fungi in cultivated and uncultivated soils in Pakistan. Entomological Research. 43: 70-78. https://doi.org/10.1111/1748-5967.12003

Supplementary material

Table S1. Experimental layout to assess the effect of *Beauveria bassiana* (Bb04) application at different concentrations on *H. armigera* under field conditions in comparison to the chemical insecticide nitenpyram.

R_3	T_4	T_1	T_5	T_3	T_6	T_2
R_2	T_5	T_6	T_1	T_2	T_4	T_3
R_1	T_6	T_2	T_3	T_4	T_1	T_5

T1: Control, T2: Water, T3: *Beauveria bassiana* strain Bb04 spore concentration 2×10^9cfu/g, T4: *Beauveria bassiana* strain Bb04 spore concentration of fungus 2×10^8 cfu/g, T5: *Beauveria bassiana* strain Bb04 spore concentration of fungus 2×10^7cfu/g, T6: chemical insecticide nitenpyram.

Table S2. Analysis of variance for the growth of different isolated entomopathogenic fungi.

SOV	DF	SS	MS	F value	p-value
Number of days for sporulation	7	22.5000	3.21429	3.21	0.0252
Error	16	16.0000	1.00000		
Total	23	38.5000			
Colony diameter on the 10th day (mm)	7	4146.70	592.385	592	0.0000
Error	16	16.00	1.000		
Total	23	4162.70			
Sporulation per plate (10^9cfu) on 10th day	7	2.3892	0.34131	0.34	0.9228
Error	16	16.0000	1.00000		
Total	23	18.3892			
Number of days taken to cover the plate	7	100.500	14.3571	14.4	0.0000
Error	16	16.0000	1.00000		
Total	23	116.500			
Fungal spore length	7	43.97	6.28190	7.77	0.004
Error	16	12.94	0.80875		
Total	23	56.91			
Fungal spore length	7	2.33167	0.33310	36.3	0.0000
Error	16	0.14667	0.00917		
Total	23	2.47833			

Table S3. Analysis of variance for the effect of entomopathogenic fungi on armyworm survival percentage.

SOV	DF	SS	MS	F value	p-value
Treatment	9	8071.91	896.879	13.50	0.00
Error	17	1132.72	66.630		
Total	26	9204.63			

Table S4. Analysis of variance for the effect of different carbon sources on *B. bassiana.*

SOV	DF	SS	MS	F value	p-value
Fungal growth on 1% carbon sources	4	0.10497	0.02624	9.09	0.0023
Error	10	0.02887	0.00289		
Total	14	0.13384			
Fungal growth on 2% carbon sources	4	0.04503	0.01126	30.7	0.00000
Error	10	0.00367	0.00037		
Total	14	0.04869			
Spore production on 1% carbon sources	4	12.0627	3.01567	38.3	0.00000
Error	10	0.7867	0.07867		
Total	14	12.8493			
Spore production on 2% carbon sources	4	17.6640	4.41600	33.6	0.00000
Error	10	1.3133	0.13133		
Total	14	18.9773			

Table S5. Analysis of variance for the effect of different nitrogen sources on *B. bassiana.*

SOV	DF	SS	MS	F value	p-value
Fungal growth on 1% nitrogen sources	4	2.88123	0.72031	6.46	0.0078
Error	10	1.11487	0.11149		
Total	14	3.99609			
Fungal growth on 2% nitrogen sources	4	7.52493	1.88123	36.3	0.0000
Error	10	0.51820	0.05182		
Total	14	8.04313			
Spore production on 1% nitrogen sources	4	13.2973	3.32433	32.0	0.0000
Error	10	1.0400	0.10400		
Total	14	14.3373			
Spore production on 2% nitrogen sources	4	96.2293	231	231	0.0000
Error	10	1.0400	0.1040		
Total	14	97.2693			

Table S6. Analysis of Variance for the Effect of *Beauveria bassiana* (Bb04) application at different concentrations on *H. armigera* under field conditions in comparison to chemical insecticide nitenpyram.

SOV	DF	SS	MS	F value	p-value
At day of 1st application	5	560.26	112.051	0.70	0.6325
Error	12	1914.74	159.562		
Total	17	2475.00			
3rd day after 1st application	5	839.12	167.824	0.88	0.5242
Error	12	2293.36	191.113		
Total	17	3132.48			
7th day after 1st application	5	7435.44	1487.09	7.59	0.0020
Error	12	2350.65	195.89		
Total	17	9786.09			
At day of 2nd application	5	5913.31	1182.66	12.3	0.0002
Error	12	1151.60	95.97		
Total	17	7064.90			
3rd day after 2nd application	5	13641.6	2728.32	49.4	0.0000
Error	12	662.2	55.18		
Total	17	14303.8			
7th day after 2nd application	5	24202.2	4840.43	71.9	0.0000
Error	12	808.0	67.34		
Total	17	25010.2			
At day of 3rd application	5	29670.5	5934.09	111	0.0000
Error	12	642.5	53.54		
Total	17	30313.0			
3rd day after 3rd application	5	29203.4	5840.68	6.93	0.0029
Error	12	10116.5	843.04		
Total	17	39319.9			
7th day after 3rd application	5	57990.0	11598.0	292	0.0000
Error	12	476.2	39.7		
Total	17	58466.2			

Table S7. Comparative analysis for the effect of *Beauveria bassiana* (Bb04) for all applications on *H. armigera* under field conditions in comparison to the chemical insecticide nitenpyram.

SOV	DF	SS	MS	F value	p-value
At day of all applications	5	19560.9	3912.17	8.43	0.0000
Error	48	22276.6	464.10		
Total	53	41837.4			
3rd day after all applications	5	31114.5	6222.90	10.9	0.0000
Error	48	27478.8	572.47		
Total	53	58593.3			
7th day after all applications	5	75932.0	15186.4	41.1	0.0000
Error	48	17747.5	369.7		
Total	53	93679.5			