Plant Protection, 09 (02) 2025. 425-438 DOI:10.33804/pp.009.02.5694

Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

ISOLATION AND IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI WITH MYCOINSECTICIDAL POTENTIAL FROM AGRICULTURAL FIELDS IN SINDH, PAKISTAN

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ARTICLE INFO

Article history
Received: 07th March, 2025
Revised: 16th June, 2025
Accepted: 25th June, 2025

Keywords
Entomopathogenic fungi
Mycoinsecticides
Biological control
Insect-pathogenic fungi
Sindh agriculture

ABSTRACT

A total of 151 samples were collected from rhizospheric soil, insect cadavers, and plant parts across 24 districts of Sindh between January and December 2023. Entomopathogenic fungi (EPF) were recovered from 29.14% of the samples (n = 44), while the remaining 70.86% (n = 107) yielded either other fungal species or no growth. Eleven EPF genera were identified, with *Beauveria* (12 isolates), *Metarhizium* (9), *Isaria* (6), *Lecanicillium* (5), and *Nomuraea* (4) being the most common; *Beauveria* was the most frequently isolated genus, accounting for 27.3% of all EPF isolates. EPF recovery was highest from soil samples (23 isolates), followed by insect cadavers (18) and plant parts (3). The highest number of isolates (6) was obtained from Khairpur district. Notably, EPF were isolated from major crops such as wheat, sugarcane, and paddy. Laboratory bioassays against mustard aphids revealed significant insecticidal potential in isolates PPL 75, PPL 81, and PPL 83, all exhibiting low LC_{50} values. Screen house trials confirmed these results, with PPL 75 (*B. bassiana*) achieving a 97.8% reduction in aphid populations and PPL 83 (*M. anisopliae*) 93.1%. Re-isolation from aphid cadavers further validated the pathogenicity of these strains. This study confirms the diversity of EPF in Sindh's agriculture and underscores the biocontrol potential of indigenous strains. Despite prior reports from Pakistan, commercial products remain absent due to methodological and developmental gaps. The findings lay a foundation for developing locally adapted EPF-based pest management strategies.

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INTRODUCTION

There is a growing demand for organic food (OF) in the global market (Peng, 2019). Rising environmental awareness has led to public concern over the indiscriminate use of toxic chemical pesticides in agriculture (Carson, 2012; Clark, 2017). Consequently, the demand for pesticide-free agricultural products has steadily increased (Lima et al., 2015). Many sectors of crop production are known for their excessive reliance on hazardous synthetic chemicals to control insect, fungal, weed, and rodent pests. Among these, chemical insecticides are particularly criticized for their harmful

effects on human health, as pesticide residues often remain in food and ultimately enter the human food chain (Castillo et al., 2000; Tariq et al., 2007).

Scientific investigations have identified several effective alternatives to chemical pesticides, including microbial pesticides, botanicals, soap-based formulations, and biological control agents such as predatory insects, bats, birds, and frogs (Lacey, 1997). The use of living organisms to manage pest populations is formally referred to as "biological control". Eilenberg et al. (2001) defined it as "the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be". This strategy also encompasses eco-friendly attractants, mechanical traps, advanced cropping systems, and the development of resistant cultivars as organic alternatives to synthetic pesticides (Steinkraus et al., 1993; Eilenberg et al., 2001).

The use of entomopathogenic fungi (EPF) in pest control dates back approximately 150 years. Their potential was first noticed in sericulture settings, where fungal infections in silkworms led farmers to collect and apply the pathogens to other crops as insecticides. The formal application of EPF was documented by Metchnikoff in Russia in 1888, when he used *Metarhizium anisopliae* against insect pests (Lord, 2005). Later, bacteria such as *Bacillus thuringiensis* (Bt) were also used successfully from the 1960s onward and became well-established bioinsecticides. The subsequent incorporation of Bt genes into crop plants represented a major advancement in the development of genetically modified organisms (GMOs). Alongside bacterial, viral, protozoan, and nematode entomopathogens, fungi have demonstrated significant potential in the development of bioinsecticides (Auld, 2002; Lacey and Shapiro-Ilan, 2008).

Mycoinsecticides, fungal agents used for insect control, are primarily based on EPF, which are gaining recognition as viable alternatives to chemical insecticides in food crop protection (Butt and Goettel, 2000; Ujjan and Shahzad, 2014). Several commercial formulations of EPF have received widespread acceptance from environmentalists and organic food producers due to their efficacy and non-toxic nature. Multinational companies are increasingly incorporating EPF formulations into their organic insecticide products, generating substantial profits (Silva et al., 2015). Mycologists and researchers continue to search for novel EPF strains to register as mycoinsecticide products, aiming to secure patents for institutional, national, and personal benefit.

In Pakistan, limited research has been conducted on EPF isolation from insect cadavers in agricultural fields. Although a few reports exist on field-based EPF isolation, these efforts have not progressed sufficiently to evaluate and commercialize them as branded mycoinsecticide products. Therefore, researchers have proposed the isolation and evaluation of indigenous, environmentally friendly EPF from rhizospheric soil, diseased insect pests, and plants (Freed et al., 2012; Akmal et al., 2013; Ujjan and Shahzad, 2014).

Two major fungal orders, Entomophthorales (Zygomycota) and Hypocreales (Ascomycota), include EPF capable of infecting a wide range of insect pests, including mites and spiders. However, species within the order Hypocreales have shown greater potential for commercial development as mycoinsecticides. Despite the fact that approximately 750 EPF species are known to infect insects in nature, only 12 have been registered and widely commercialized. Prominent among these are *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii*, and *Isaria fumosorosea*, which are considered valuable bioinsecticide commodities (Faria and Wraight, 2007; Ujjan and Shahzad, 2007). EPF offer distinct advantages over bacterial agents, especially in their modes of action against sap-sucking pests (Bateman et al., 1993; Butt and Goettel, 2000; Butt et al., 2016).

The proposed research aims to screen and characterize potent EPF strains for potential commercial development as mycoinsecticides. This study involves the systematic collection of diseased insect pests from irrigated crop fields in Sindh, isolation of EPF from rhizospheric soil and plant surfaces, pure culturing, taxonomic identification, bioassays, and the selection of the most effective EPF candidates for potential field trials.

MATERIALS AND METHODS

Field visit and sampling

Sampling was conducted using standard materials and methods as described in established research manuals and reference books. The following materials were employed: polyethylene bags, test tubes, Eppendorf tubes, forceps, a Garmin eTrex 10 GPS device, test tube aspirators (AB-98-11d), a locally fabricated soil auger,

and transport vehicles. Insect and plant samples were collected directly using sterile forceps and placed into sterile tubes or vials. Soil samples were collected from a depth of 5 inches at agricultural sites, following protocols outlined by Goettel and Inglis (1997). Samples were collected from 24 districts of Sindh for the isolation and recovery of EPF. The samples were obtained from rhizospheric soil, insect cadavers, and plant parts. Each collection site was geo-referenced using GPS coordinates. Sampling was conducted throughout the year, from January to December 2023 (Figure 1). Extensive sampling was conducted across different seasons to account for variations in fungal diversity associated with seasonal and humidity changes (Ahmed, 2013). Infected insects were collected using sterilized forceps and placed in sterile, dry, airtight glass vials (BKMAN Airtight Thread Cap Transparent Amber, 3-60 ml) or Eppendorf tubes (Kisker, 2 ml). Samples were transported in insulated containers (AUCMA, 11L) maintained at <10°C and delivered to the laboratory within 24 h, where they were stored at 4°C. Plant parts surrounding the habitat of diseased or dead insects were also collected and transported under the same conditions. A standardized field data form was used to record sample details and assign collection numbers.

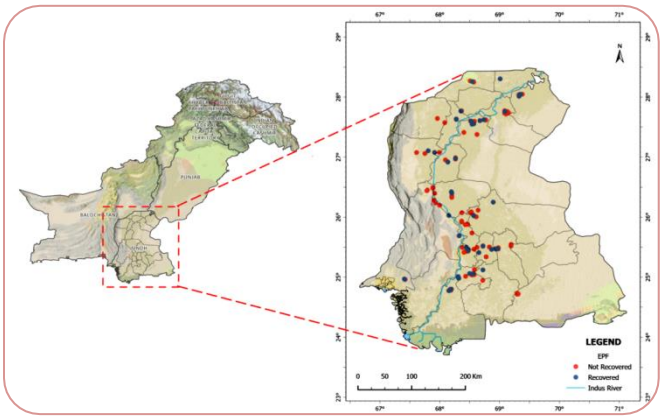

Figure 1. Map showing the study area and sites in agricultural fields of Sindh from which EPF were recovered, highlighting regions of Indus River-irrigated agriculture.

Randomized sampling

Sampling sites were randomly selected using an agricultural map of Sindh, based on crop type and locality. A total of 24 districts were included. Monthly visits were made to each district throughout 2023 for random crop sampling. Collected samples were transported to the Plants' Pathogens, Pests, and Protection Laboratory (PL), University of Sindh, following the procedures described above. Samples were stored in thermopile containers at <4°C for less than 6 h, following the guidelines by Hoben (2016) (Figure 2).

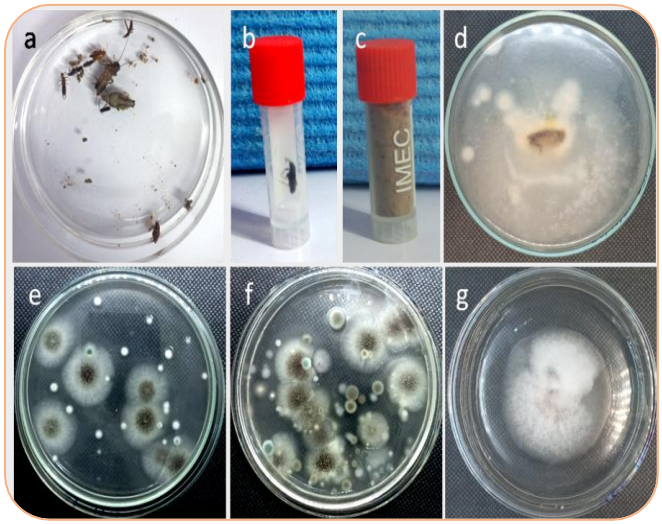

Figure 2. Workflow for EPF isolation: sample processing (a-c), recovery from soil using the dilution method (e-f) or from surface-sterilized insect cadavers (g), and pure culturing on specialized media.

Isolation of EPF from samples

Insect specimens displaying symptoms of infection were collected from 24 agricultural districts across Sindh, Pakistan. Both live and dead insects exhibiting signs of fungal infection, such as abnormal positioning on host plants or visible mycelial growth, were collected using sterile forceps, needles, and aspirators. Each specimen was placed in a sterilized screw-cap glass or plastic tube (1.5 × 0.3 inches) and stored in shaded, humid containers.

Samples were transported to the laboratory within 3 to 18 h. Cadavers showing visible fungal growth were examined under a stereomicroscope (Ningbo ZTX-3S-C3) to record fungal colony features and conidial color. Hyphal structures were transferred onto microscope slides for preliminary identification and were also cultured on potato dextrose agar (PDA) plates (Figure 2d-f).

Surface sterilization of insect cadavers was performed using 1% sodium hypochlorite (Wuhan Snow Medical Disinfectant) for 1 min, followed by three rinses in sterile distilled water. Samples were dried on sterile blotting paper and placed on Sabouraud Dextrose Agar (SDA) amended with dodine (20 ppm) and antibiotics

(penicillin 200 ppm + streptomycin 20 ppm) to promote selective growth of entomopathogenic fungi. Fungal growth was observed between 5 to 7 days post-inoculation.

Moreover, humid chambers were prepared by placing sterile, colourless blotting paper moistened with 3 ml distilled water inside Petri dishes, which were autoclaved at 121°C and 15 psi for 15 min. Surface-sterilized insects were incubated at room temperature, and fungal emergence was monitored daily. Hyphal growth from cadavers was transferred to SDA, PDA, and YEMA media for culturing. After confirming pure fungal colonies, hyphae were transferred to SDA slants and stored at 10°C for 15 to 30 days (Lacey, 2012). Host plant samples were similarly screened for entomopathogenic fungi.

Soil samples were serially diluted from 10^{-1} to 10^{-5}, and 01 ml of each dilution was spread on SDA media plates (amended as above). Each fungal isolate was assigned an accession number and recorded with information on collection date, isolation date, host, source, location, and collector.

Mycological media preparation

Potato Dextrose Agar (PDA) was prepared by boiling 200 g of peeled potato cubes in 500 ml of water, filtering the extract, and mixing with 20 g dextrose and 15 g agar (Merck Millipore, CAS 9002-18-0 101614). The volume was adjusted to 1000 ml, dispensed into containers, sealed with cotton plugs, and autoclaved at 121°C and 15 psi for 15 min.

Potato Sucrose Agar (PSA) was prepared similarly to PDA, replacing dextrose with 20 g sucrose.

Sabouraud Dextrose Agar (SDA) was prepared by dissolving 10 g peptone, 40 g dextrose, and 15 g agar in 1000 ml distilled water, heated to 70°C, and autoclaved at 121°C and 15 psi for 15 min.

Yeast Extract Mannitol Agar (YEMA) consisted of 10 g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.2 g NaCl, 1 g yeast extract, and 20 g agar in 1000 ml distilled water. The mixture was boiled, dispensed into containers, and autoclaved.

Chitin Agar (CA) was prepared using 4 g chitin (Sigma-Aldrich, 1398-61-4), 0.7 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.3 g KH_2PO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.001 g $MnCl_2 \cdot 4H_2O$, 0.001 g $ZnSO_4 \cdot 7H_2O$, and 20 g agar in 1000 ml distilled water. The mixture was autoclaved and amended with sterile additives before pouring into 90 mm sterilised glass Petri plates (Anumbra).

Media additives for selectivity

To inhibit non-target microbes and enhance entomopathogenic fungi recovery (particularly Hypocrealean fungi), dodine (20 ppm) was added. After autoclaving and cooling, antibiotics (penicillin 200 ppm and streptomycin 20 ppm, Shijiazhuang G-House Pharma Ltd) were added using sterile syringes.

Fungal identification

Fine hyphal filaments were picked using a minutron needle or insect pin and mounted in Lactophenol Cotton Blue or aceto-orcein (Sigma-Aldrich) on clean glass slides. Samples were gently crushed, covered with a coverslip, and examined under a compound microscope (Nikon ECLIPSE Si). Fungal identification was based on morphological features with reference to established taxonomic keys (Barnett and Hunter, 1972; Domsch et al., 1980; Watanabe, 2002; Humber, 2007, 2012; Samson et al., 2013).

Laboratory bioassays

Mustard aphids were reared on mustard plants and used in fungal bioassays. Spore suspensions of EPF were prepared in 0.2% Tween 20 (Sigma-Aldrich) and quantified using a haemocytometer (Bitesize Bio). Different spore concentrations were applied to 10 mustard aphids in Petri dish-based bioassay chambers, following protocols described by Lacey (2012), Butt and Goettel (2000), and Gautam and Avasthi (2019).

Insect mortality in various bioassays was recorded after 7 days. Lethal concentrations (LC) and lethal times (LT) were derived from the data. The bioassay results were analyzed using Probit regression analysis in SPSS (IBM-SPSS 26) to assess dose-time mortality (LT and LC). Laboratory experiments were conducted in three replicates and repeated twice to ensure statistical reliability (Dewey and Lu, 1959; Kuehl and Mead, 1990).

Spore cultivation

Spores of EPF were harvested from 15-day-old cultures grown on appropriate solid media. To collect spores, 5 ml of sterile distilled water containing 0.02% (v/v) Tween 20 was added to the culture surface. The conidia were gently dislodged using a sterile spatula, and the resulting suspension was transferred to a sterile test tube. The final volume was adjusted to 10 ml with the same Tween 20 solution. A hemocytometer was used to estimate spore concentration. Spore counts were conducted microscopically, and the suspension was standardized through serial dilutions (Goettel and Inglis, 1997).

To prepare the serial dilutions, the spore suspension was mixed with 10 ml of 0.02% Tween 80 solution in the first test tube. A series of seven test tubes containing 09 ml of the same solution were prepared. From the first tube, 01 ml was transferred sequentially to each subsequent tube. The spore concentration per ml was then determined using a hemocytometer (Waksman and Fred, 1922; Goettel and Inglis, 1997).

Insect rearing

Insects were reared on mustard host plants grown in 6×8" earthen pots filled with a 4:1 mixture of loamy soil and humified cow dung. The pots were maintained under a 10:14 (Light:Dark) photoperiod. Seedlings were placed inside 8×8" chambers enclosed in fine white muslin cloth supported by a steel rod framework, under natural light conditions in the screen house at the Institute of Plant Sciences, University of Sindh. The plants were irrigated and fertilized regularly.

Healthy adult insects were introduced to the plants. Once heavy infestation was observed, plants were transferred to the laboratory for insect collection. Infested surfaces were rinsed with 0.01% sodium hypochlorite and washed with sterile distilled water. Insects at specific life stages were carefully transferred into bioassay chambers using a camel hairbrush (size 0) and forceps.

Bioassay chambers

Young host plant leaves were surface-sterilized with 0.01% sodium hypochlorite for 2 min, followed by rinsing with sterilized tap water. A thin layer of sterilized, water-soaked cotton was wrapped around the petiole to maintain leaf turgidity. Each leaf was placed in a sterilized 09 cm diameter glass Petri dish. Fifteen test insects were introduced per leaf, following the procedure described by Goettel and Inglis (1997).

Artificial infection

A known number of insects were introduced into each bioassay chamber. One ml spore suspension of EPF was applied using a 26-gauge (0.25 mm) BD™ insulin syringe. The suspension, harvested from a 15-day-old EPF culture in sterile water, was drawn into the syringe along with air. Using a micro-spray technique, 0.1 ml of the suspension was gently expelled onto each insect.

Each EPF strain was applied at concentrations of 1×10^3, 1×10^4, 1×10^5, 1×10^6, and 1×10^7 spores per bioassay chamber (9 cm diameter; 63.6 cm^2). Treatments were replicated five times. A control group was treated with 0.01% Tween 20 solution. Mortality was calculated using Abbott's formula, comparing treated groups with the control. Probit regression analysis was used to determine the most probable lethal dose at constant time to mortality.

Mortality calculations

EPF-treated insects were observed daily for signs of reduced mobility or death. The number of dead insects was recorded for each treatment. Each treatment was replicated five times. Observations were tabulated, including days after treatment, spore concentrations, and the number of live and dead insects. Percent mortality was calculated and corrected using Abbott's formula for a homogeneous population (Abbott, 1987).

Correction Formulas

Abbott's corrected Mortality (%)

$$\text{Mortality } \% = \frac{t - c}{100 - c} \times 100$$

Where t = percent mortality in the treatment; c = percent mortality in the control.

Henderson-Tilton Mortality (%)

$$\text{Mortality } \% = 1 - \frac{C1 \times T2}{C2 \times T1} \times 100$$

Where, the C1 = No. of insects before treatment in control, C2 = No. of insects after treatment in control, T1 = No. of insects before treatment and, T2 = No. of insects after treatment. The data were entered into IBM-SPSS 26 for further analysis of lethal concentrations using probit regression.

Screen house bioassays

Host plants were grown in earthen pots filled with sterile sandy loam soil. The experiment was conducted at the Greenhouse Facility, Institute of Plant Sciences, University of Sindh, Jamshoro.

Mustard seeds were sown in November 2024 at a depth of 5 cm, followed by irrigation and fertilization according to agronomic recommendations. The Canola cultivar "Oscar" was selected for screen house trials. In December 2024, five mustard aphid nymphs were introduced onto each plant.

EPF spores were suspended in sterile water with 0.3% Tween 20. The final concentration was adjusted to $1.0\times10^7 \pm 0.05$ spores/ml. The suspension was sprayed onto aphid-infested plants. Initial aphid infestation was recorded before spraying, and final infestation levels were assessed 15 days post-application. Each treatment was replicated five times and isolated with 01 mm plastic netting, spaced 3 meters apart to prevent cross-contamination. Control plants were treated with sterile

water containing 0.3% Tween 20.

Mortality percentages were calculated using the Henderson-Tilton formula. Insect cadavers were examined under a microscope and cultured on PDA plates to confirm EPF recovery. The data were analyzed in IBM-SPSS 26.

Statistical analysis

EPF isolates were recorded based on their host or habitat (plant, insect, or soil), along with information such as date, area, crop, and weather conditions. Data were analyzed using IBM-SPSS 26 for ANOVA and dissimilarity tests.

Sample collection sites were geo-referenced using GPS, and EPF isolate frequency and dominance were assessed across crops and regions.

Mortality data were analyzed using probit regression to estimate the median lethal concentration (LC_{50}), keeping time constant at 5 days for laboratory bioassays and 15 days for screen house bioassays (Ashford and Sowden, 1970).

RESULTS

Sample collection

EPF were successfully recovered from 29.14% of the samples (n = 44), while 70.86% (n = 107) either yielded non-target fungi or showed no fungal recovery. A total of 151 samples were collected, out of which 44 contained EPF and were successfully cultured on laboratory media (Table 1).

EPF Isolation

From the collected samples, 11 EPF genera were identified. A total of 12 *Beauveria* spp. isolates were recovered, followed by *Metarhizium* spp. (9), *Paecilomyces* spp. (6), and *Nomuraea* spp. (4), with other entomopathogenic fungi (EPF) recovered in smaller numbers from 24 districts of Sindh (Table 2). Among the EPF, *Beauveria* was the most prevalent, accounting for 27.3% of recoveries (Figure 3), indicating its widespread presence in Sindh fields. *Metarhizium* was the second most frequent (20.5%), followed by *Isaria* (13.6%), *Lecanicillium* (11.4%), *Nomuraea* (9.1%), *Trichoderma* (6.8%), and *Purpureocillium*, *Penicillium*, *Pandora*, *Paecilomyces*, and *Gliocladium* (each at 2.3%).

The proportions of EPF isolates obtained from different sources, namely insects, plants, and soil, across the province are presented in Figure 3. The highest number of EPF isolates were recovered from soil samples (23) followed by insects (18) and plants (3).

Isolates were recovered from most collection districts, with Khairpur yielding 6 isolates, Tando Muhammad Khan 5, Ghotki and Hyderabad 4 each, Thatta and Jamshoro 2 each, and one isolate each from Badin, Jacobabad, Karachi, Kashmore, and Sukkur. No isolates were recovered from Matiari, Qamber, Sajawal, Shikarpur, Tharparkar, and Umerkot (Table 3). Major crops yielding EPF isolates included wheat (16), sugarcane (7), paddy (5), chilli (3), and barseen (2), while other crops yielded fewer than two isolates (Figure 3).

Table 1. Isolation counts of EPF species/samples.

Sr. No.	Species (EPF)	Count
1	*G. roseum*	1
2	*I. farinosa*	1
3	*Isaria sp.*	1
4	*P. chrysogenium*	1
5	*P. lilacinum*	1
6	*P. neoaphidis*	1
7	*P. variotii*	1
8	*T. harzianum*	1
9	*T. pseudokoningii*	1
10	*Trichoderma sp.*	1
11	*I. fumosorosea*	4
12	*N. rileyi*	4
13	*L. lecanii*	5
14	*M. anisopliae*	9
15	*B. bassiana*	12
16	NRF/NA*	107
Total	151	

Table 2. Diversity and frequency of EPF genera isolated from collected samples.

Sr. No.	EPF	No. of Isolates
1	*Beauveria*	12
2	*Metarhizium*	09
3	*Isaria*	06
4	*Lecanicillium*	05
5	*Nomuraea*	04
6	*Trichoderma*	03
7	*Gliocladium*	01
8	*Paecilomyces*	01
9	*Pandora*	01
10	*Purpureocillium*	01
11	*Penicillium*	01
12	Total	44

Table 3. Recovery of EPF isolates across districts of Sindh.

Sr. No.	District	No. of Isolates Recovered
1	Khairpur	06
2	Tando Muhammad Khan	05
3	Ghotki	04
4	Hyderabad	04
5	Thatta	02
6	Jamshoro	02
7	Badin	01
8	Jacobabad	01
9	Karachi	01
10	Kashmore	01
11	Sukkur	01
12	Matiari	0
13	Qamber	0
14	Sajawal	0
15	Shikarpur	0
16	Tharparkar	0
17	Umerkot	0

Laboratory bioassays

The isolates demonstrated significant insecticidal activity in laboratory bioassays. Live mustard aphids were exposed to EPF isolates in bioassay chambers, with control chambers containing no EPF. Comparative data between treated and untreated aphids revealed the mycoinsecticidal potency of the EPF isolates after seven days.

Among the isolates, PPL83 exhibited the highest mycoinsecticidal potential, with an LC_{50} value of 2.0×10^5 ($p < 0.05$). Several other isolates, PPL75, PPL81, PPL85, PPL18, PPL09, PPL16, PPL10, and PPL52, also showed strong insecticidal effects, with LC_{50} values ranging from 2.4×10^5 to 3.7×10^5 ($p < 0.05$).

Another group of isolates, including PPL105, PPL115, PPL56, PPL38, and PPL64, exhibited moderate lethality, with LC_{50} values ranging from 3.419×10^5 to 6.324×10^5 ($p < 0.05$) (Table 4).

Overall, the effective insecticidal potential was primarily observed in isolates belonging to three genera: *Metarhizium*, *Beauveria*, and *Paecilomyces*. The EPF isolates were further characterized based on colony morphology, microscopic features, and the biometric analysis of spores and phialides.

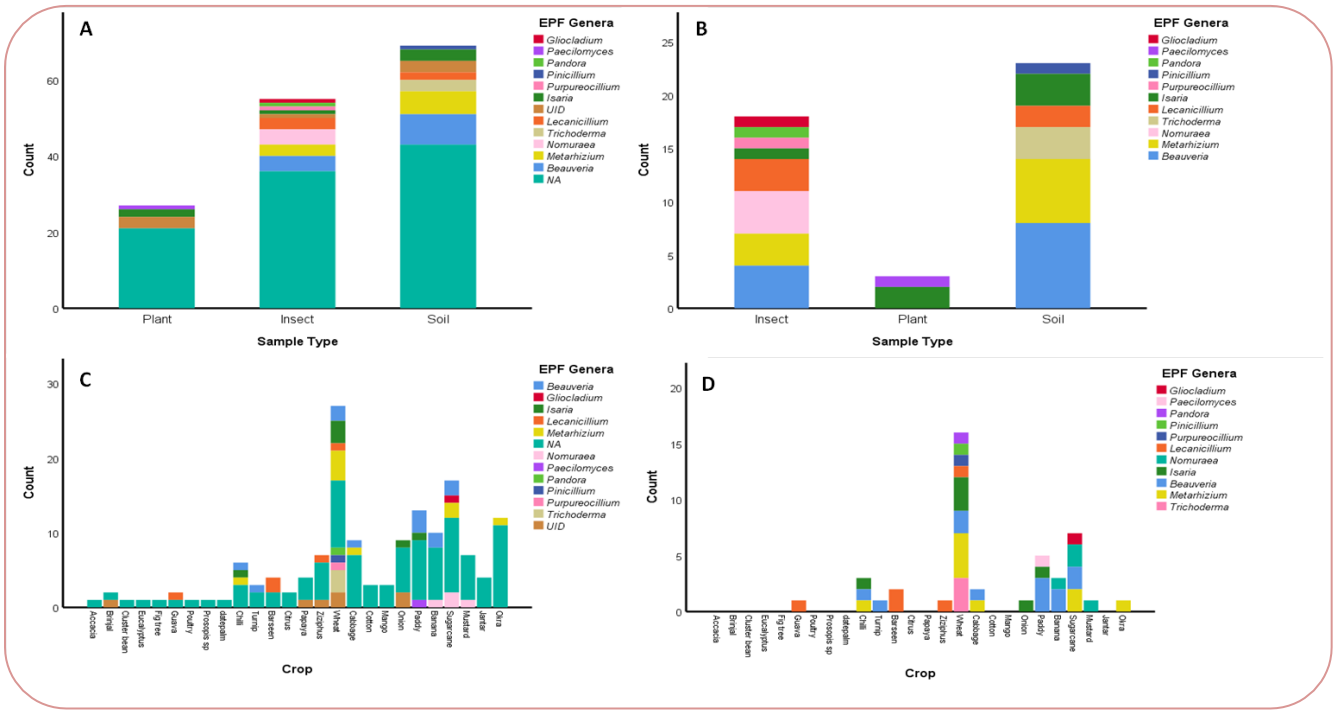

Figure 3. Proportion of EPF isolates from insect, plant, and soil sources across the province: (A) All samples, (B) EPF-positive samples only, (C) Crop-wise distribution of EPF genera and unrecovered samples, and (D) Breakdown of EPF sources.

Table 4. Toxicity of EPF isolates to mustard aphids based on Probit analysis of median lethal concentration (LC_{50}) values.

Sr. No.	Isolate		EPF species	LC_{50}	Pearson Goodness-of-Fit Test			Y (Estimate)		
					χ^2	df	Sig.	Conc.	Intercept	Sig.
1	PPL	83	*M. anisopliae*	1.69E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
2	PPL	75	*B. bassiana*	2.44E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
3	PPL	81	*M. anisopliae.*	2.44E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
4	PPL	85	*B. bassiana*	2.44E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
5	PPL	18	*M. anisopliae*	3.11E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
6	PPL	9	*B. bassiana*	3.18E+05	23.680	3	0.000[a]	0.748	-4.240	0.000
7	PPL	16	*P. variotii*	3.21E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
8	PPL	10	*B. bassiana*	3.31E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
9	PPL	52	*B. bassiana*	3.41E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
10	PPL	105	*M. anisopliae*	3.41E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
11	PPL	115	*B. bassiana*	3.51E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
12	PPL	56	*M. anisopliae*	3.71E+05	34.275	3	0.000[a]	0.798	-4.423	0.000
13	PPL	38	*M. anisopliae*	6.32E+05	17.029	3	0.001[a]	0.706	-4.094	0.000
14	PPL	64	*M. anisopliae*	6.32E+05	17.029	3	0.001[a]	0.706	-4.094	0.000
15	PPL	114	*M. anisopliae*	1.10E+06	9.433	3	0.024[a]	0.637	-3.873	0.000
16	PPL	60	*B. bassiana*	1.12E+06	9.433	3	0.024[a]	0.637	-3.873	0.000
17	PPL	37	*I. farinosa*	1.13E+06	9.433	3	0.024[a]	0.637	-3.873	0.000
18	PPL	31	*B. bassiana*	1.20E+06	9.433	3	0.024[a]	0.637	-3.873	0.000
19	PPL	107	*L. lecanii*	1.20E+06	9.433	3	0.024[a]	0.637	-3.873	0.000
20	PPL	66	*B. bassiana*	1.89E+06	9.433	3	0.024[a]	0.637	-3.873	0.000
21	PPL	104	*L. lecanii*	5.53E+06	3.210	3	0.360[a]	0.533	-3.596	0.000
22	PPL	11	*B. bassiana*	5.63E+06	3.210	3	0.360[a]	0.533	-3.596	0.000
23	PPL	102	*B. bassiana*	5.63E+06	3.210	3	0.360[a]	0.533	-3.596	0.000
24	PPL	84	*I. fumosorosea.*	6.17E+06	3.042	3	0.385[a]	0.528	-3.586	0.000
25	PPL	77	*P. lilacinum*	1.21E+07	1.858	3	0.602[a]	0.489	-3.510	0.000
26	PPL	19	*B. bassiana*	1.51E+07	1.858	3	0.602[a]	0.489	-3.510	0.000
27	PPL	40	*I. fumosorosea*	1.51E+07	1.858	3	0.602[a]	0.489	-3.510	0.000
28	PPL	74	*UID*	1.51E+07	1.858	3	0.602[a]	0.489	-3.510	0.000
29	PPL	82	*T. pseudokoningii*	1.51E+07	1.858	3	0.602[a]	0.489	-3.510	0.000
30	PPL	106	*Trichoderma sp.*	1.51E+07	1.858	3	0.602[a]	0.489	-3.510	0.000
31	PPL	63	*G. roseum*	2.38E+07	1.394	3	0.707[a]	0.468	-3.477	0.000
32	PPL	43	*P. chrysogenum*	2.68E+07	1.394	3	0.707[a]	0.468	-3.477	0.000
33	PPL	5	*P. fumosorosea*	5.11E+07	1.031	3	0.794[a]	0.448	-3.452	0.000
34	PPL	108	*L. lecanii*	5.11E+07	1.031	3	0.794[a]	0.448	-3.452	0.000
35	PPL	23	*UID*	5.71E+07	1.031	3	0.794[a]	0.448	-3.452	0.000
36	PPL	41	*T. harzianum*	5.88E+07	0.968	3	0.809[a]	0.444	-3.448	0.000
37	PPL	61	*N. rileyi*	6.68E+07	0.909	3	0.823[a]	0.440	-3.444	0.000
38	PPL	59	*I. fumosorosea*	6.78E+07	0.909	3	0.823[a]	0.440	-3.444	0.000
39	PPL	62	*N. rileyi*	6.78E+07	0.909	3	0.823[a]	0.440	-3.444	0.000
40	PPL	99	*L. lecanii*	1.07E+08	0.747	3	0.862[a]	0.428	-3.434	0.000
41	PPL	2	*L. lecanii*	2.51E+08	0.526	3	0.913[a]	0.407	-3.423	0.000
42	PPL	94	*M. anisopliae*	2.51E+08	0.526	3	0.913[a]	0.407	-3.423	0.000
43	PPL	36	*UID*	6.87E+08	0.355	3	0.949[a]	0.387	-3.421	0.000
44	PPL	27	*UID*	6.97E+08	0.355	3	0.949[a]	0.387	-3.421	0.000
45	PPL	17	*Isaria sp.*	2.46E+09	0.226	3	0.973[a]	0.365	-3.431	0.000
46	PPL	48	*UID*	2.46E+09	0.226	3	0.973[a]	0.365	-3.431	0.000
47	PPL	51	*N. rileyi*	2.46E+09	0.226	3	0.973[a]	0.365	-3.431	0.000
48	PPL	58	*UID*	2.46E+09	0.226	3	0.973[a]	0.365	-3.431	0.000
49	PPL	46	*N. rileyi*	1.27E+10	0.131	3	0.988[a]	0.342	-3.457	0.000
50	PPL	80	*P. neoaphidis*	2.43E+11	0.053	3	0.997[a]	0.310	-3.528	0.000
51	PPL	92	*UID*	2.43E+11	0.053	3	0.997[a]	0.310	-3.528	0.000

[a] Since the significance level is greater than 0.150, no heterogeneity factor is used in the calculation of confidence limits. UID refers to the unidentified epf or others.

Screen house bioassays

EPF isolates were selected for screen house bioassays against mustard aphids. Insect infestation was assessed 15 days post-treatment. Isolates that exhibited LC_{50} values ≤ 1.0×10^5 in laboratory bioassays were selected for the screen house trials. A total of 14 isolates were prepared for spray application.

Among them, *B. bassiana* PPL75 demonstrated the highest virulence, reducing the aphid population by 97.8% compared to the untreated control. This was followed by *M. anisopliae* PPL83 (93.1%), PPL81 (81.5%), PPL18 (64.2%), *B. bassiana* PPL85 (61.5%), PPL9 (58.5%), PPL10 (57.9%), *P. variotii* PPL16 (55.9%), *M. anisopliae* PPL56 (53.8%), *B. bassiana* PPL52 (51.8%), PPL115 (47.9%), *M. anisopliae* PPL105 (43.4%), PPL38 (33.5%), and PPL64, which showed the lowest virulence at 12.6%.

The effect of the 14 EPF isolates on mustard aphid (*Lipaphis erysimi*) mortality was statistically significant. One-way ANOVA revealed a highly significant difference among fungal treatments ($F_{13, 20}$ = 1581.23, P < 0.001). The large variation in mortality rates between treatment groups, coupled with minimal variation within groups, indicates considerable differences in the pathogenicity of the EPF isolates. These findings demonstrate that certain fungal isolates were markedly more effective against mustard aphids under screen house conditions (Figure 4A).

Recovery of EPF from treated insect cadavers

Recovery trends mirrored the observed mortality rates. *B. bassiana* PPL75 not only showed the highest virulence but also the highest recovery rate (81.2%). PPL83 and PPL81 also showed strong recovery at 77.3% and 67.7%, respectively. Moderate recovery was observed for isolates such as PPL9, PPL10, and PPL16, ranging from 28.0% to 29.2%. Isolates PPL56, PPL52, PPL115, PPL105, PPL38, and PPL64 exhibited lower recovery rates, ranging from 16.2% down to 3.8%.

Statistical analysis showed significant differences in the recovery rates of aphids treated with the different EPF isolates. One-way ANOVA revealed a highly significant difference among treatments ($F_{10, 20}$ = 1944.94, P < 0.001). The high F-value and low within-group variance indicate consistent differences among isolates in their ability to persist and suppress aphid resurgence. These results suggest that certain EPF isolates were more effective in preventing aphid population rebound post-infection (Figure 4B).

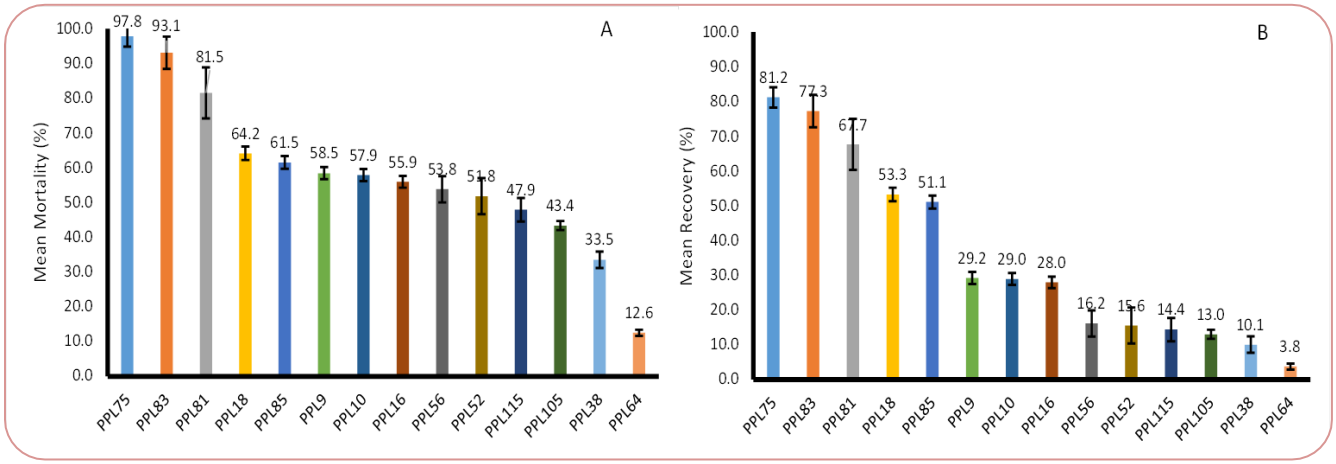

Figure 4. Mortality rate of mustard aphids (A) and recovery of EPF from cadavers or morbid aphids (B), 15 days after application of EPF suspension (1.0×10^7 spores/ml) on infested mustard plants in screenhouse bioassays.

Although the isolates showed varying levels of virulence and recovery, the most efficient candidates for mycoinsecticide development are PPL75 (*B. bassiana*), PPL83 (*M. anisopliae*), and PPL81 (*M. anisopliae*). These isolates caused devastating effects on the aphid populations, with infestations almost completely eradicated 15 days post-treatment (Figure 5 and 6).

The efficient EPF isolates exhibited significant virulence characteristics consistent with established entomopathogenic fungi manuals and guidelines (Hajek and Goettel, 2000). The fungal cultures are preserved at the Culture Collections Facility of the Plant Pathogens, Pests, and Protection Laboratory (PL), Institute of Plant Sciences, University of Sindh, Jamshoro.

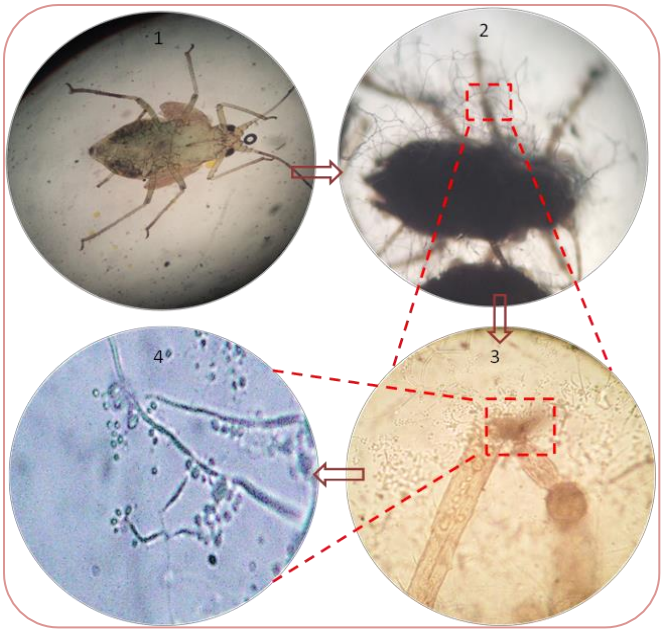

Figure 5. (1) A healthy mustard aphid infesting a mustard plant before the EPF (PPL75) *B. bassiana* bioassay; (2) the aphid succumbed to death seven days after infection by *B. bassiana* spores; (3) infected body appendages showing evidence of the isolate's efficacy under *in vivo* conditions; (4) confirmation of the fungal infection through microscopy (400x) showing recovery of the bioassay strain.

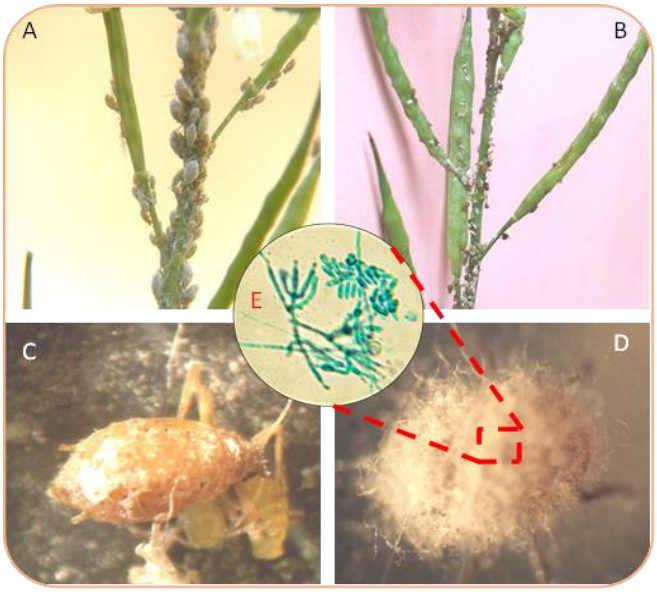

Figure 6. Aphid infestation on mustard plants: (A) healthy aphid population on untreated (control) plant; (B) aphid cadavers on plant treated with EPF isolate PPL83 (*M. anisopliae*); (C) dehydrated aphid cadavers on plant; (D) fungal mycosis on mustard aphids under *in vivo* conditions; and (E) magnified micrograph of the fungal isolate at 1000×.

DISCUSSION

There is ample research from Pakistan on the development of mycoinsecticides; however, no product has yet been registered for commercial use in the field based on indigenous EPF strains. Some researchers have successfully collected fungal isolates with significant virulence against insect pests of various crops. Numerous records exist on the isolation of indigenous fungal strains in Pakistan from different samples (Qazi and Khachatourians, 2005; Bilal et al., 2012; Ahmed, 2013; Wakil et al., 2013, 2014; Qayyum et al., 2024). Wakil et al. (2013) reported the natural occurrence of EPF in Pakistan, identifying 210 soil isolates with the following distribution: *B. bassiana* (8), *B. brongniartii* (2), *M. anisopliae* (5), and other fungi (12). This represents a relatively low frequency compared to the present study, likely due to overlooked methodologies in EPF recovery during media formulation, which may have led to contamination. Nevertheless, the work by Wakil et al. (2013, 2014) remains a notable contribution to EPF research in Pakistan (Akmal et al., 2013).

Extensive EPF surveys have been conducted nationwide, yet no indigenous EPF-based product has reached commercialization. The present study aims to identify a set of robust EPF isolates that meets commercial development criteria. Frequent recovery of *Beauveria* spp. from field samples has been previously reported (Wakil et al., 2013, 2014). Similarly, Akmal et al. (2013) reported EPF isolation from cotton field soils in Multan, with recovery rates ranging from 2% to 11%, where *M. anisopliae* showed the highest frequency. Our findings are consistent with these earlier reports, and other researchers have also noted the high frequency of *M. anisopliae* in agricultural soils (Ahmed, 2013).

In Sindh province, a separate study reported EPF isolates dominated by *Isaria* spp. (11), *Metarhizium* spp. (9), *Lecanicillium* sp. (6), and *Beauveria* spp. (1) (Ahmed, 2013). Interestingly, fungi associated with grasshoppers in Sindh were not typical EPF, indicating a possible absence of natural fungal checks in these pest populations (Anwar et al., 2012; Kumar et al., 2014). Our current study confirms the presence of EPF in Sindh's agro-ecosystems, highlighting their potential role in insect pest control and contributing positively to ecological balance. There is a strong need to classify EPF as a valuable natural resource, which could be utilized as inundative mycoinsecticides, especially in areas like arid Sindh where natural EPF populations are low. This study

helps fill the knowledge gap on EPF prevalence in the agricultural zones of Sindh.

In Europe, EPF are reported to occur in 71.7% of field soils, with *B. bassiana* accounting for 42.6%, *M. anisopliae* 7.3%, and mixed genera 21.7% (Quesada-Moraga et al., 2007). In contrast, the present study recorded EPF occurrence below 30%, suggesting a significantly lower prevalence compared to European data. These differences may be attributed to climatic variations such as humidity, temperature, UV intensity, rainfall, and cloud cover. The climate of India, being more comparable to Pakistan than Europe, presents EPF occurrence rates of 71.1% for *B. bassiana* and 2.03% for *M. anisopliae*, which still differ from our findings (Thakur and Sandhu, 2010). This highlights the diverse distribution of EPF in Sindh's fields and suggests the need for targeted studies to explore the low EPF presence, which could hypothetically contribute to higher insect pest infestations reported in the region. Therefore, there is an urgent need to augment indigenous EPF populations in crop fields to establish a natural balance between pests and fungal pathogens. EPF offer strong prospects for development as mycoinsecticides, particularly in integrated pest management strategies.

The present study aligns with previous reports on the virulence of EPF against insect pests such as the mustard aphid. Ujjan and Shahzad (2012) found strain PDRL526 (*M. anisopliae*) to be highly virulent against mustard aphid under laboratory conditions (LT_{50} = 4.32 × 10^6 spores/ml). Strains PDRL711 (*M. anisopliae*, LT_{50} = 3.51 × 10^8) and PDRL812 (*Paecilomyces lilacinus*, LT_{50} = 4.01 × 10^5) also showed high efficacy. Ahmed (2013) similarly reported the effectiveness of local strains in bioassay trials. However, most studies indicate that EPF tend to lose virulence under field conditions (Goettel and Inglis, 1997). Therefore, we propose to evaluate EPF isolates under *in vivo* conditions to assess their sustained virulence against the mustard aphid.

EPF identification is not inherently difficult for experienced mycologists, although it is labor-intensive to determine the specific species and varieties. This study initially used established resources, articles, and websites to identify the isolates. Humber (2012) provided detailed micrographic and taxonomic descriptions, which were followed in this study. The "Atlas of Entomopathogenic Fungi" (Samson et al., 2013) was also instrumental in describing the isolates. Additional identification keys and compendia used include Barnett and Hunter (1972), Domsch et al. (2007), and Watanabe (2002). Although morphological identification remains the fundamental method, molecular techniques provide precise strain-level identification. In this study, basic identification was followed by molecular confirmation of the most promising isolates, as this step is generally required for developing commercially viable mycoinsecticides. The EPF strains were stored using a combination of glycerol, casein, and agar solution at 4°C for long-term viability (Feng et al., 1994).

An EPF isolate cannot be deemed commercially viable for mycoinsecticide production based solely on laboratory bioassays. These assays serve as controlled tools for selecting the most promising isolates from a larger pool. Comprehensive evaluation must also consider the interaction of the EPF strain with biotic and abiotic factors, including its environment, target insect, and host plant. EPF bioefficacy is largely dependent on environmental conditions. When these factors align, the EPF is considered efficient (Butt and Goettel, 2000). We tested the EPF isolates under screen house conditions to evaluate their environmental resilience and ability to induce insect mycosis in mustard aphid infestations. Although screen houses do not fully replicate field conditions, they expose isolates to moderate environmental stresses. The isolates were screened for effectiveness, comparable to commercial EPF products (Faria and Wraight, 2007). An isolate is considered effective if it reduces the insect population and can be re-isolated from cadavers, confirming its pathogenicity. In our study, EPF isolates were successfully recovered from treated insects, indicating their effectiveness.

Based on these results, we have selected five efficient isolates for further field bioassays, mass production, formulation development, molecular characterization, and long-term storage for future use. Despite numerous EPF isolation studies in Pakistan, no product has yet achieved global recognition (Ujjan and Shahzad, 2012; Batta, 2016; Qayyum et al., 2024). This study presents a dataset of 14 EPF isolates, among which two, PPL75 (*B. bassiana*) and PPL83 (*M. anisopliae*), have shown high efficacy in bioassay regimes and are thus proposed for further development into mycoinsecticides.

ACKNOWLEDGEMENT

Special thanks are extended to Dr. Saleem Shahzad (Department of Agriculture and Agribusiness Management, University of Karachi) and Dr. Abdul Mubeen Lodhi (Sindh Agriculture University, Tandojam)

for their valuable guidance in the identification and characterization of the entomopathogenic isolates; their support is deeply appreciated.

AUTHORS' CONTRIBUTIONS

AAU and MAK designed the study; KM prepared the materials, collected and analysed the data; SN helped in insect rearing; AAU supervised the studies and wrote the manuscript; All the authors proofread and approved the final manuscript.

RESEARCH FUNDING

This research was solely funded by the Sindh Higher Education Commission (SHEC) under the Project No: SHEC/SRSP, A, AE-3/83/2022-23.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265-267.

Ahmed, A., 2013. Use of entomopathogenic fungi in biological control of cotton mealybug (*Phenacoccus solenopsis*) and mustard aphid (*Lipaphis erysimi*). PhD Thesis, University of Karachi.

Akmal, M., Freed, S., Malik, M.N., Gul, H.T., 2013. Efficacy of *Beauveria bassiana* (Deuteromycotina: Hypomycetes) against different aphid species under laboratory conditions. Pakistan Journal of Zoology 45, 71-78.

Anwar, W., Khan, S.N., Haider, M.S., 2012. Occurrence of insect-associated fungi in hot arid zone, Pakistan. African Journal of Microbiology Research 6, 6878-6883.

Ashford, J.R., Sowden, R.R., 1970. Multi-variate probit analysis. Biometrics 26, 535-546.

Auld, B., 2002. Fungi as biocontrol agents: progress, problems and potential. Plant Pathology 51, 299-299.

Barnett, H.L., Hunter, B.B., 1972. Illustrated Genera of Imperfect Fungi, 4th ed. Burgess Publishing Co., Minneapolis, Mycologia.

Bateman, R.P., Carey, M., Moore, D., Prior, C., 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. Annals of Applied Biology 122, 145-152.

Batta, Y.A., 2016. Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Science and Technology 26, 1171-1183.

Bilal, H., Hassan, S.A., Khan, I.A., 2012. Isolation and efficacy of entomopathogenic fungus (*Metarhizium anisopliae*) for the control of *Aedes albopictus* Skuse larvae: suspected dengue vector in Pakistan. Asian Pacific Journal of Tropical Biomedicine 2, 298-300.

Butt, T.M., Coates, C.J., Dubovskiy, I.M., Ratcliffe, N.A., 2016. Entomopathogenic fungi: new insights into host-pathogen interactions. Advances in Genetics 94, 307-364.

Butt, T.M., Goettel, M.S., 2000. Bioassays of entomogenous fungi. In: Navon, A., Ascher, K.R.S. (Eds.), Bioassays of Entomopathogenic Microbes and Nematodes. CABI, Wallingford, pp. 141-195.

Carson, R., 2012. Silent Spring. In: Gleadle, R. (Ed.), Key Readings in Journalism 53, 290-298.

Castillo, L.E., Ruepert, C., Solis, E., 2000. Pesticide residues in the aquatic environment of banana plantations: areas in the north Atlantic zone of Costa Rica. Environmental Toxicology and Chemistry 19, 1942-1950.

Clark, J.F.M., 2017. Pesticides, pollution and the UK's silent spring, 1963-1964: poison in the garden of England. Notes and Records 71, 297-327.

Dewey, D.R., Lu, K.H., 1959. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal 51, 515-518.

Domsch, K.H., Gams, W., Anderson, T.H., 1980. Compendium of Soil Fungi, Vols. 1 and 2.

Domsch, K.H., Gams, W., Anderson, T.H., 2007. Compendium of Soil Fungi: Taxonomically Revised by W. Gams. IHW, Eching, 53, 12-26.

Eilenberg, J., Hajek, A., Lomer, C., 2001. Suggestions for unifying the terminology in biological control. BioControl 46, 387-400.

Faria, M.R. de, Wraight, S.P., 2007. Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biological

Control 43, 237-256.

Feng, M.G., Khachatourians, G.G., Poprawski, T.J., 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Science and Technology 4, 3-34.

Freed, S., Saleem, M.A., Khan, M.B., Naeem, M., 2012. Prevalence and effectiveness of *Metarhizium anisopliae* against *Spodoptera exigua* (Lepidoptera: Noctuidae) in southern Punjab, Pakistan. Pakistan Journal of Zoology 44, 753.

Gautam, A.K., Avasthi, S., 2019. Methods in fungal biology: a manual of laboratory protocols. Journal of Plant Protection Research 53, 12-26.

Goettel, M.S., Inglis, G.D., 1997. Fungi: Hyphomycetes. In: Lacey, L.A. (Ed.), Manual of Techniques in Insect Pathology. Elsevier, pp. 213-249.

Hajek, A.E., Goettel, M.S., 2000. Guidelines for evaluating effects of entomopathogens on non-target organisms. In: Lacey, L.A. (Ed.), Field Manual of Techniques in Invertebrate Pathology, pp. 847-868.

Hoben, H., 2016. Handbook for Rhizobia: Methods in Legume-Rhizobium Technology. Journal of Plant Protection Research 53, 511.

Humber, R.A., 2007. Recent phylogenetically based reclassifications of fungal pathogens of invertebrates. ARSEF, New York, 5 pp. Available at: http://www.sipweb.org/fungi/humber.pdf

Humber, R.A., 2012. Identification of entomopathogenic fungi. In: Lacey, L.A. (Ed.), Manual of Techniques in Invertebrate Pathology. Elsevier, pp. 151-187.

Kuehl, R.O., Mead, R., 1990. The design of experiments: Statistical principles for practical applications. Biometrics 46, 876.

Kumar, S., Riffat, S., Wagan, M.S., 2014. Entomopathogenic fungi in population of Acridid grasshopper from Sindh, Pakistan. International Journal of Advanced Research 2, 227-231.

Lacey, L.A., 1997. Manual of Techniques in Insect Pathology. Academic Press.

Lacey, L.A., 2012. Manual of Techniques in Invertebrate Pathology. Academic Press.

Lacey, L.A., Shapiro-Ilan, D.I., 2008. Microbial control of insect pests in temperate orchard systems: Potential for incorporation into IPM. Annual Review of Entomology 53, 121-144.

Lima, G., Sanzani, S., De Curtis, F., Ippolito, A., 2015. Biological control of postharvest diseases. Journal of Plant Protection Research 53, 65-88.

Lord, J.C., 2005. From Metchnikoff to Monsanto and beyond: The path of microbial control. Journal of Invertebrate Pathology 89, 19-29.

Peng, M., 2019. The growing market of organic foods: Impact on the US and global economy. In: Safety and Practice for Organic Food. Elsevier, pp. 3-22.

Qayyum, M.A., Saeed, S., Wakil, W., Sharif, U., Taha, H., Shahid, Z., Khalid, A., Hameed, O., Yasin, M., Nawaz, A., 2024. Entomopathogenic fungi: Prospects and challenges. In: Entomopathogenic Fungi: Prospects and Challenges, pp. 57-79.

Qazi, S., Khachatourians, G., 2005. Insect pests of Pakistan and their management practices: Prospects for the use of entomopathogenic fungi. Biopesticides International 1, 1-13.

Quesada-Moraga, E., Navas-Cortés, J.A., Maranhao, E.A.A., Ortiz-Urquiza, A., Santiago-Álvarez, C., 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research 111, 947-966.

Samson, R.A., Evans, H.C., Latgé, J.-P., 2013. Atlas of Entomopathogenic Fungi. Springer Science & Business Media.

Silva, A., Alves, R., Lima, E., Lima, V., 2015. Bioformulations in pest control - A review. Annual Research & Review in Biology 5, 535-543.

Steinkraus, D.C., Mueller, A.J., Humber, R.A., 1993. *Furia virescens* (Thaxter) Humber (Zygomycetes: Entomophthoraceae) infections in the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae) in Arkansas with notes on other natural enemies. Journal of Entomological Science 28, 376-386.

Tariq, M.I., Afzal, S., Hussain, I., Sultana, N., 2007. Pesticides exposure in Pakistan: A review. Environment International 33, 1107-1122.

Thakur, R., Sandhu, S.S., 2010. Distribution, occurrence and natural invertebrate hosts of indigenous entomopathogenic fungi of Central India. Indian Journal of Microbiology 50, 89-96.

Ujjan, A.A., Shahzad, S., 2007. Pathogenicity of *Metarhizium anisopliae* var. *acridum* strains on pink hibiscus mealy bug (*Maconellicoccus hirsutus*) affecting cotton crop. Pakistan Journal of Botany 39, 967-973.

Ujjan, A.A., Shahzad, S., 2012. Use of entomopathogenic fungi for the control of mustard aphid (*Lipaphis*

erysimi) on canola (*Brassica napus* L.). Pakistan Journal of Botany 44, 2081-2086.

Ujjan, A.A., Shahzad, S., 2014. Insecticidal potential of *Beauveria bassiana* strain PDRL1187 and Imidacloprid to mustard aphid (*Lipaphis erysimi*) under field conditions. Pakistan Journal of Zoology 46, 1277-1281.

Wakil, W., Ghazanfar, M.U., Riasat, T., Kwon, Y.J., Qayyum, M.A., Yasin, M., 2013. Occurrence and diversity of entomopathogenic fungi in cultivated and uncultivated soils in Pakistan. Entomological Research 43, 70-78.

Wakil, W., Ghazanfar, M.U., Yasin, M., 2014. Naturally occurring entomopathogenic fungi infecting stored grain insect species in Punjab, Pakistan. Journal of Insect Science 14, 182.

Waksman, S.A., Fred, E.B., 1922. A tentative outline of the plate method for determining the number of micro-organisms in the soil. Soil Science 14, 27-28.

Watanabe, T., 2002. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, 2nd ed. 53, 1-487.