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Scanning Electron Microscopic Analysis and Biocontrol Potential of Entomopathogenic Fungi Against Grasshoppers

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Authors' Contribution
SK conceived and designed the study, conducted fieldwork and laboratory experiments. RS supervised the research project, provided critical revisions to the manuscript, and approved the final version for submission.

Key words
***Entomopathogenic fungi, Aspergillus,* Grasshopper, Mortality, Biocontrol, SEM, IPM**

ABSTRACT

The present study evaluated the pathogenicity of five native strains of entomopathogenic fungi, including *Aspergillus niger, A. flavus, A. fumigatus*, and two unidentified fungal strains, against various pest species of grasshoppers. To confirm fungal identification and assess structural characteristics, scanning electron microscopy (SEM) analysis was conducted. Significant differences were observed among the strains in terms of phialide coloration, spore morphology, and growth patterns. Elemental composition determined through SEM spectrum acquisition revealed that in *A. niger*, the normal weight percentage of oxygen (O_2) was the highest at 56.19±17.5%, followed by carbon (C) at 42.60±13.1%, while sodium (Na) was present in the least amount (1.21 ±0.1%). For *A. flavus*, carbon content was highest at 52.33±16.1, followed by oxygen at 46.84±14.5%, with sodium again recorded in minimal quantity. In *A. fumigatus*, oxygen was dominant at 54.61±17.1%, followed by carbon at 43.92±13.6%, with minor concentrations of sodium (0.92%), sulfur (0.35%), and phosphorus (0.20%) detected. Biologically, insect mortality was most pronounced in treatments involving *Aspergillus spp*., which caused rapid declines in grasshopper populations, particularly among nymphal stages (N1 to N3), with only a few individuals surviving. The highest mortality rate was recorded on the first day of treatment [$F_{0.48} = 84.65$, $P < 0.05$], followed by day four [$F_{0.35} = 61.96$, $P < 0.05$] and day two [$F_{0.27} = 48.00$, $P < 0.05$]. This study is the first to report the efficacy of *Aspergillus* spp. against both nymphal and adult stages of grasshoppers, highlighting their potential as effective biological control agents within integrated pest management strategies.

INTRODUCTION

Diseases caused by entomopathogenic fungi in insect fauna are widespread and have been extensively studied worldwide (Ferron, 1985; Goettel *et al*., 1990; Assaf *et al*., 2011). These fungi can rapidly decimate insect populations through spectacular epizootics. In natural field conditions, most insect populations are highly susceptible to such pathogenic fungi. Since insect orders like Diptera and Orthoptera are among the most damaging to agriculture, entomopathogenic fungi should be strategically employed against them. When fungal pathogens are abundant in the field, they significantly reduce insect populations and often contribute to their natural regulation (Samson *et al*., 1988). Entomopathogenic fungi can lethally affect all developmental stages of grasshoppers, from the embryonic stage to adulthood. For instance, Hemiptera overwinter in plant root zones and are often affected by these fungi (Kubatova and Dvorak, 2005). Kilic (1976) reported that *Beauveria bassiana* can kill up to 80% of Sunn pests. While several entomopathogenic fungal species such as *Beauveria*, *Aspergillus*, and *Metarhizium* are commercially available for the control of flies, aphids, and thrips (Upadhyay, 2003), their application as biopesticides against grasshoppers has not yet been widely explored. Therefore, the present study aims to expand our understanding of the occurrence and pathogenic potential of *Aspergillus* species for controlling grasshopper populations in the field. The microbial agent studied proved to be highly effective in reducing major pest species (Table I). This study suggests that introducing a novel biocontrol agent one not previously encountered by the target pest may offer a more effective and sustainable approach to pest management. Recent studies have renewed interest in the use of entomopathogenic fungi as environmentally sustainable alternatives to chemical pesticides, particularly in the context of increasing resistance and ecological concerns.

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Table I. Important pest species of Acrididae occurring in Sindh.

Species
Subfamily: Acridinae
Acrida exaltata (Walker, 1859)
A. gigantea (Herbst, 1786)
Duroniella laticornis (Krauss, 1909)
Gelastorhinus semipictus (Walker, 1870)
Phlaeoba infumata Brunner von Wattenwyl, 1893
P. tenebrosa Walker, 1871
Truxalis exmia exmia Eichwald, 1830
T. fitzgeraldi Drish, 1950
Subfamily: Calliptaminae
Acorypha glaucopsis (Walker, 1870)
Sphodromerus undulatus undulatus (Kirby, 1914)
Subfamily: Gomphocerinae
Chorthippus indus Uvarov, 1942
Ch. dorsatus (Zetterstedt, 1821)
Gonista rotundata (Uvarov, 1933)
Ochrilidia geniculata (Bolivar, 1913)
Oxypterna afghana Ramme, 1952
Subfamily: Hemiacridinae
Hieroglyphus banian (Fabricius, 1798)
H. nigrorepletus Bolivar, 1912
H. oryzivorus Carl, 1916
H. perpolita (Uvarov, 1933)
Spathosternum prasiniferum (Walker, 1871)
Subfamily: Oedipodinae
Acrotylus humbertianus Saussure, 1884
A. longipes longipes (Charpentier 1845)
Aiolopus thalassinus thalassinus (Fabricius, 1781)
Hilethera aeolopoides (Uvarov, 1922)
Locusta migratoria (Linnaeus, 1758)
Oedaleus rosescens Uvarov, 1942
O. senegalensis (Krauss, 1877)
Trilophidia annulata (Thunberg, 1815)
Subfamily: Oxyinae
Oxya bidentata (Willemse, 1925)
O. fuscovittata (Marschall, 1836)
O. hyla hyla Serville, 1831
O. velox (Fabricius, 1787)

Fungal strains, formulations and application methods have progressively improved field performance of entomopathogenic fungi against a range of insect pests (Mascarin and Jaronski, 2016). In addition, Shahid *et al*. (2023). With over 92% mortality in a semi-arid field, Bankola *et al*. (2023) showed the efficacy of Aspergillus and Metarhizium species against Orthopteran pests (grasshoppers) with the advantages of low non-target effects. This support is pivotal in a modern integrated pest management (IPM) approach, especially in areas where ecological imbalance has been exacerbated due to synthetic insecticide misuse. Thus presently, the study provides timely evidence that Aspergillus species could be useful organisms as potential biological control agents of grasshoppers in agroecosystems undergoing pest outbreak situations.

According to Mascarin and Jaronski (2016), advances in fungal strain selection, formulation technologies, and application methods have significantly improved the field efficacy of entomopathogenic fungi against various insect pests. Furthermore, a study by Shahid *et al*. (2012) demonstrated the successful application of *Aspergillus* and *Metarhizium* species under semi-arid conditions for the suppression of Orthopteran pests, including grasshoppers, with notable mortality rates and minimal non-target effects. These findings support the integration of entomopathogenic fungi into modern integrated pest management (IPM) programs, especially in regions facing ecological imbalance due to overuse of synthetic insecticides. Therefore, the current study contributes timely evidence that *Aspergillus* species can serve as viable biological control agents against grasshoppers in agroecosystems facing pest outbreaks.

MATERIALS AND METHODS

Insect sampling

Grasshoppers (both nymphs and adults) were collected from various districts of Sindh (Table I). Specimens were captured using a sweep net (diameter: 8.89 cm; length: 50.8 cm). Larger individuals were collected using forceps, while first instar nymphs were picked by hand. The collected insects were transported to the laboratory, where they were placed in two rearing cages of different dimensions (42 cm × 30 cm and 35 cm × 32.5 cm). Groups of 50 individuals were housed per cage and provided with fresh Zea mays leaves as food. This methodology, with slight modifications, was adapted from Prior *et al*. (1995) and Sultana *et al*. (2013) and Jamil and Sultana (2025). For species identification, the classification scheme developed by Sultana and Wagan (2008, 2015) was followed.

Collection of infected specimens

Grasshoppers showing clear symptoms of fungal infection (mycoses) were selectively collected. These

individuals exhibited sluggish behavior and minimal resistance upon capture. Infected specimens were reared separately in cages and jars to closely observe the progression of fungal growth on the host body. Observations were made for a duration of 24–72 h.

Insect rearing

Various species of Acrididae were grouped into sets of approximately 50 individuals, regardless of age, sex, or developmental stage. The collections were maintained under laboratory conditions in wooden cages, with temperatures ranging from 28 ± 2°C to 41 ± 2°C and relative humidity (RH) from 26.5% to 60.5%. All developmental stages of field-collected grasshoppers were maintained at the Entomology and Bio-Control Research (EBCRL), Department of Zoology, University of Sindh, Jamshoro (25°-23/N, 68°-24/E).

Fungal isolation and sporulation test

Sporulating entomopathogenic fungi were isolated in pure culture using Sabouraud Dextrose Agar (SDA), a medium favorable for fungal growth. The isolated cultures were formulated into a coconut oil-based suspension. To ensure uniform dispersion and breakage of conidial chains, the formulation was sonicated for 60 sec. Conidial concentration was then quantified using a hemocytometer, following standardized procedures adopted and modified from Poinar and Thomas (1984), Kumar *et al*. (2013), and more recently from Qayyum *et al*. (2020) and Ullah *et al*. (2021). Identification of Aspergillus species was conducted based on morphological characteristics and culture characteristics according to the recent taxonomic descriptions of Samson *et al*. (2017), the databases of the Westerdijk Fungal Biodiversity Institute.

Observations under scanning electron microscopy (SEM-EDS)

Fungal spores were subjected to SEM coupled with EDS for elemental composition at the Centre for Pure and Applied Geology, University of Sindh, Jamshoro. Three known species as *Aspergillus niger, A. flavus*, and *A. fumigatus*, as well as two unidentified fungal isolates (Uk FI and Uk FII), were included to analyze five fungal samples. Infected specimens in both isolation and co-isolation were killed, and fungal spores were subsequently recovered from cadavers using the protocol of Kumar *et al*. (2014) subsequently improved by Sharma *et al*. (2019) and Ahmad *et al*. (2022). Each sample was kept under natural sunlight 12–14 h to obtain full dehydration. Dried spore samples were then mounted for SEM-EDS analysis, to assess qualitative and quantitative elemental composition. The data on the mineral uptake and surface morphology of entomopathogenic fungi has been discussed.

The experimental procedure for SEM-EDS analysis was started by manually cutting fungal core chips with distinct structures, followed by stepwise mounting on SEM sample stubs with conductive double-sided carbon adhesive tape. SEM images of all samples were taken within the chamber of a JEOL JSM-6490LV SEM fitted with a Bruker Energy Dispersive X-ray Spectroscopy (EDS) unit for elemental composition analysis. The sample was left inside the SEM, and it took 15–20 min to get required vacuum level achieved inside the SEM. After a stable vacuum was shown on the interface of the system, specific portions of each sample were chosen for imaging and elemental analysis using EDS. Each Aspergillus sample was mounted in a consistent manner on the conductive carbon tape, labeled with a unique identifier, and analyzed in sequence. High-resolution magnified images were captured, and EDS analysis was conducted to determine the elemental composition of each fungal sample. Optimal SEM operational parameters were configured to ensure fine focusing and desired magnification levels. Once an ideal magnification typically around ×80 was achieved, the samples were subjected to both qualitative and quantitative elemental analysis. Elemental composition was determined by examining the characteristic X-ray peaks produced during EDS, where peak height indicated the presence of specific elements. Finally, the results were compiled in both tabular and graphical formats to clearly present the qualitative identities and quantitative proportions of elements found in the fungal spores.

RESULTS

During the present study, five subfamilies of Acrididae namely *Acridinae*, *Calliptaminae*, *Gomphocerinae*, *Hemiacridinae*, *Oedipodinae*, and *Oxyinae* were represented by 32 pest species of grasshoppers (Table I). These were treated with various entomopathogenic fungi. Fungal identification and elemental analysis were carried out using SEM coupled with EDS (Table II). Spectrum acquisition for *Aspergillus niger* revealed that oxygen (O_2) had the highest normal weight percentage at 56.19±17.5%, followed by carbon (C) at 42.60±13.1%, while sodium (Na) was present in the lowest amount at 1.21±0.1%. In the case of *Aspergillus flavus*, the highest elemental composition was observed for carbon (C) at 52.33±16.1%, followed by oxygen (O_2) at 46.84±14.5%. Sodium was again detected in the lowest proportion. Similarly, *Aspergillus fumigatus* showed a greater percentage of oxygen (O_2) at 54.61±17.1%, followed by carbon (C) at 43.92±13.6%. Other elements detected included sodium

(Na) at 0.92%, sulfur (S) at 0.35% and phosphorus (P) at 0.20%, all with minimal error values (Table I; Fig. 1, 2). Two unidentified fungal isolates (Uk FI and Uk FII) were also analyzed. In Uk FI, carbon (C) content was the highest at 62.82±19.2%, followed by oxygen (O_2) at 36.82 ±11.4%, while sodium was detected in trace amounts. Uk FII presented five elements, with carbon (C) again being dominant at 54.00±16.6%, followed by oxygen (O_2) at 43.53±13.6%. The lowest values were recorded for sulfur (S) and sodium (Na) at 0.79% and 0.1%, respectively. Overall, the elemental composition of the three known *Aspergillus* species and the two unidentified fungi showed significant variation, indicating possible species-specific physiological traits (Table II; Figs. 1, 2). Biological assays showed that insects treated with *Aspergillus* species exhibited rapid mortality, especially in early developmental stages (N1 to N3). Most immature stages succumbed, with only a few individuals surviving the fungal exposure. Mortality was highest on Day 1, showing statistically significant results [F0.48=84.65, P<0.05], followed by Day 4 [F0.35=61.96, P<0.05], and Day 2 [F0.27=48.00,P<0.05]. The lowest mortality was recorded on Day 3, but it was still statistically significant [F0.17=30.54, P<0.05] (Table II).

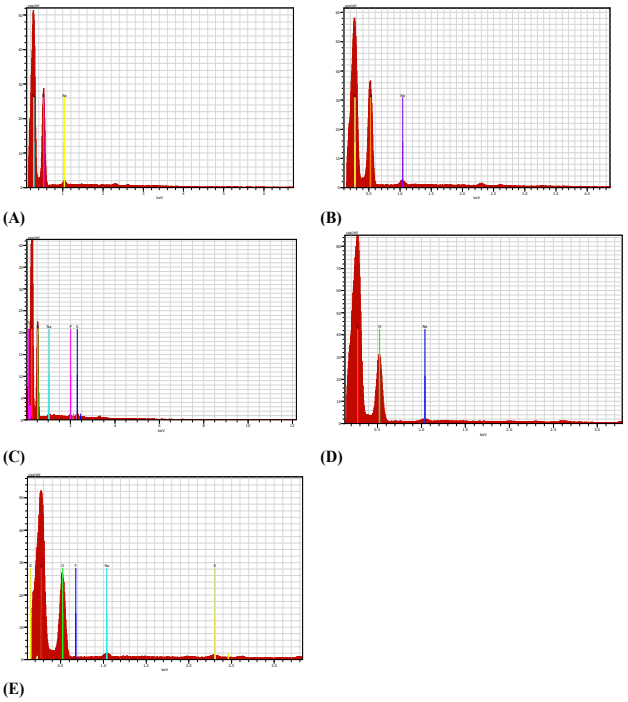

Fig. 1. Element concentrations under scanning electron microscope (SEM): A, *Aspergillus niger*; B, *A. flavus*; C, *A. fumigatus*; D, unknown Fungi I; E, unknown Fungi II.

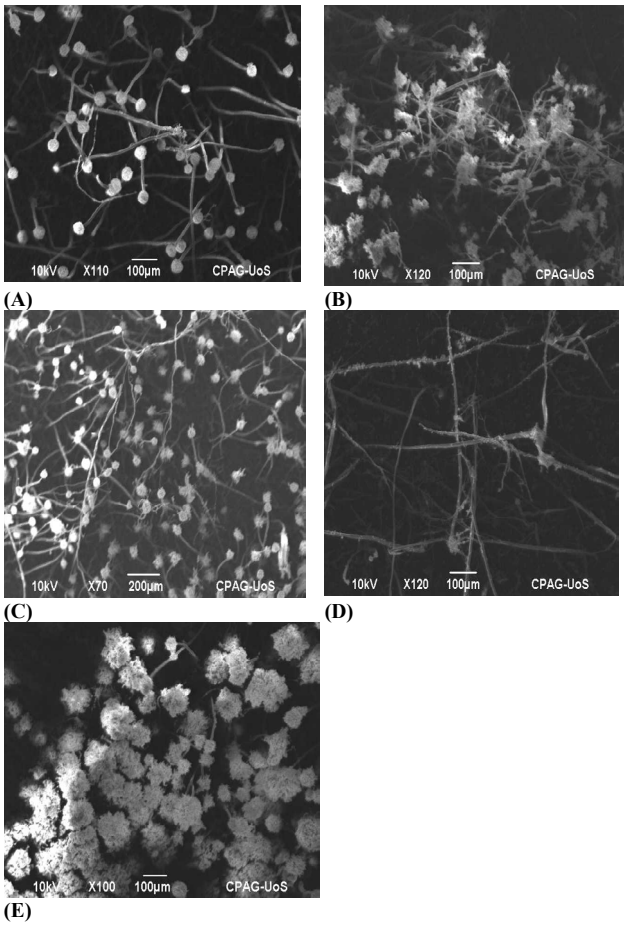

Fig. 2. Electron microscopy: A, *Aspergillus niger*; B, *A. flavus*; C, *A. fumigatus*; D, unknown Fungi I; E, unknown Fungi II.

Mortality trends and pathogenic effects

In the control replicates, the mortality ratio for nymphal stages (N4–N6) was highest on Day 2 [F10.7=18.33, P<0.05], followed by Day 4 [F4.20=7.85, P<0.05] and Day 3 [F3.77=6.11, P<0.05]. Conversely, the lowest mortality was recorded on Day 1 [F0.48=84.65, P<0.05] (Table III). Throughout the experiment, it was consistently observed that fungal-infected individuals showed reduced feeding activity and suffered from multiple pathological symptoms. Regarding nymphal populations kept in large cages and treated with conidial suspensions prepared in water, the highest mortality was observed on Day 6 [F0.82=43.99, P<0.05] (Table IV). However, the mortality was statistically non-significant on Day 2 [F8.5=14.84, P<0.05] and Day 3 [F7.25=13.09,P<0.05], while significantly lower mortality was noted on Day 5 [F3.32=6.11, P<0.05] (Table IV). In adult *Acrididae*, the maximum mortality occurred on Day 7 [F13.7=23.56, P<0.05], followed by Day 6 [F12.5=21.82,

Table II. Spectrum acquisition under SEM.

Element	Series	unn. C (wt. %)	Norm. C (wt. %)	Atom. C	Error (%)
Aspergillus niger					
Carbon (C)	K-series	42.60[b]	42.60[b]	49.88[a]	13.1[b]
Oxygen (O_2)	K-series	56.19[a]	56.19[a]	49.88[a]	17.5[a]
Sodium (Na)	K-series	1.21[c]	1.21[c]	0.74[c]	0.1[c]
	Total	100.00	100.00	100.00	
Aspergillus flavus					
Carbon (C)	K-series	52.33[a]	52.33[a]	59.88[a]	16.1[a]
Oxygen (O_2)	K-series	46.84[b]	46.84[b]	39.88[b]	14.5[b]
Sodium (Na)	K-series	0.83[c]	0.83[c]	0.49[c]	0.1[c]
	Total	100.00	100.00	100.00	
Aspergillus fumigatus					
Carbon (C)	K-series	43.92[b]	43.92[b]	51.31[a]	13.6[b]
Oxygen (O_2)	K-series	54.61[a]	54.61[a]	47.89[b]	17.1[a]
Sulfur (S)	K-series	0.35[d]	0.35[d]	0.15[d]	0.0[d]
Sodium (Na)	K-series	0.92[c]	0.92[c]	0.56[c]	0.1[c]
Phosphorus (P)	K-series	0.20[d]	0.20[d]	0.09[d]	0.0[d]
	Total	100.00	100.00	100.00	
Unknown Fungi I.					
Carbon (C)	K-series	62.82[a]	62.82[a]	69.30[a]	19.2[a]
Oxygen (O_2)	K-series	36.82[b]	36.82[b]	30.49[b]	11.4[b]
Sodium (Na)	K-series	0.36[c]	0.36[c]	0.21[c]	0.1[c]
	Total	100.00	100.00	100.00	
Unknown Fungi II					
Carbon (C)	K-series	54.00[a]	54.00[a]	69.38[a]	16.6[a]
Oxygen (O_2)	K-series	43.53[b]	43.53[b]	30.14[b]	13.6[b]
Fluorine (F)	K-series	1.17[c]	1.17[c]	0.84[c]	0.5[d]
Sodium (Na)	K-series	0.52[e]	0.52[e]	69.31[a]	0.1[c]
Sulfur (S)	K-series	0.79[d]	0.79[d]	30.34[b]	0.1[c]
	Total	100.00	100.00	100.00	

P<0.05] (Table V). In contrast, mortality was at its lowest on Day 1 [F0.44=77.67, P<0.05], followed by Day 3 [F0.77=35.26, P<0.05] . Mortality on Days 4 and 5 was statistically non-significant (Table V). Additionally, a significant difference was observed in the cumulative fecal output of infected insects compared to control groups. Among various treatments, the H_2O-based formulation of conidia showed the most pronounced impact on adult mortality, with a peak observed on Day 8 [F1.00=2.62, P<0.05]. Mortality was also significantly higher on Day 2 [F0.02=4.36, P<0.05], though lower than the peak, and least on Day 1 [F0.06=11.34, P<0.05]. However, from Day 3 to Day 7, the differences in mortality were statistically non-significant (Table VI).

DISCUSSION

Entomopathogenic fungi (EPFs) are increasingly recognized for their wide-ranging potential in Integrated Pest Management (IPM) due to their eco-friendly, host-specific, and biodegradable nature. These microbial agents can effectively target and control various insect pests, including grasshoppers, with minimal impact on non-target organisms and the environment (Sánchez-Ramos *et al*., 2022). In the present study, high mortality rates were observed in acridid populations, and susceptibility to fungal infection was closely linked to the host's developmental stage. Early instars (N1–N3) experienced significantly higher mortality than the older stages, which supports the results of stage-specific efficacy of EPFs Wakil *et al*. (2019). All fungal isolates tested showed efficacy, especially *Aspergillus* species against grasshoppers but varied with life stage. The differences we observe are likely due to structural and physiological defenses at later development stages, with thicker cuticles and more activated immunity (Fernandes *et al*., 2021). Under SEM imaging, the conidia showed considerable morphological diversity, shape, ontogeny and in pigmentation. Conidia varied from monocellular to multicellular and appeared in various shapes including globose, ovoid, cylindrical, and spirally coiled with colours from hyaline to melanised dark spores. These features are important for fungal classification and pathogenicity as melanin has been correlated with higher virulence and resistance to unfavorable environmental conditions (Rangel *et al*., 2020). Saccardo's conventional classification scheme based on color and morphology of the conidia is of historical interest but is now seen as restricted. Molecular approaches have been given priority over traditional mycological methods as they are vital for refining species boundaries, which results in improvement of taxonomic resolution (Ramanpreet *et al*., 2012; Kumar *et al*., 2022; Sultana *et al*., 2021). Here, we show that the differentiation based on SEM corroborates the integrated taxonomic approach we have performed. Magalhães *et al*. (2001) stated in support of our results, the use of *Metarhizium anisopliae* var was successfully demonstrated by Wraight *et al*. (2001). A review of the use of *Metarhizium acridum* as a biopesticide for grasshopper control in Brazil, although noted limitations including short storage life and variable field performance. Studies done recently, though, are trying to overcome this barrier with better formulations and cold storage (Qayyum *et al*., 2020; Ullah *et al*., 2023). We also noted reduced feeding and movement, reproductive abnormalities such as behavioral

Table III. Mortality of Acridid (Nymphs) population cultured in small jars under laboratory conditions (after treatment of *Aspergillus* oil formation).

Treatments	Days of observation (Mean ± SE)						
	1st	2nd	3rd	4th	5th	6th	7th
Nymphs stages 1st to 3rd							
A. flavus	0.45±0.23^{c}	0.35±0.21^{a}	0.20±0.21^{a}	0.8±0.23^{a}	0.00±0.00	0.00±0.00	0.00±0.00
A. fumigatus	0.62±0.01^{a}	0.22±0.01^{c}	0.16±0.01^{b}	0.2±0.01^{c}	0.00±0.00	0.00±0.00	0.00±0.00
A. niger	0.55±0.2^{b}	0.32±0.23^{b}	0.13±0.1^{c}	0.00±0.00^{d}	0.00±0.00	0.00±0.00	0.00±0.00
Control	0.3±0.1^{d}	0.2±0.1^{d}	0.2±0.23^{a}	0.4±0.2^{b}	0.00±0.00	0.00±0.00	0.00±0.00
$F_{(0.05)}$	$_{(0.48)}$84.65	$_{(0.27)}$48.00	$_{(0.17)}$30.54	$_{(0.35)}$61.96	-------	-------	-------
Nymphs Stages 4th to 6th							
A. flavus	0.29±0.1^{c}	42±0.2^{a}	13±0.2^{a}	16±0.1^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A.fumigatus	0.43±0.1^{b}	0.38±0.1^{c}	0.7±0.32^{d}	0.12±0.2^{d}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A.niger	0.62±0.2^{a}	0.28±0.1^{d}	0.8±0.2^{c}	0.2±0.1^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Control	0.6±0.1^{a}	0.4±0.3^{b}	0.6±0.2^{b}	0.5±0.3^{b}	0.7±0.1^{a}	0.6±0.1^{a}	0.5±0.1^{a}
$F_{(0.05)}$	$_{(0.48)}$84.65	$_{(10.7)}$18.33	$_{(3.77)}$06.11	$_{(4.20)}$07.85	-------	-------	-------

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

Table IV. Mortality of Acridid (Nymphs) population treated with conidial concentration in H_2O cultured maintained in the large cage.

Treatments	Days of observation (Mean ± SE)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. flavus	13±0.1^{c}	11±0.2^{b}	7±0.1^{c}	6±0.2^{b}	5±0.2^{a}	2±0.1^{a}	6±0.2^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A. fumigatus	15±0.1^{b}	12±0.1^{a}	9±0.2^{b}	7±0.2^{a}	5±0.1^{a}	1.0±0.1^{b}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
A. niger	21±0.1^{a}	7±0.2^{c}	10±0.1^{a}	7±0.2^{a}	3±0.1^{b}	0.2±0.1^{c}	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
Control	0.2±0.1^{d}	4±0.2^{d}	3±0.1^{d}	6±0.1^{b}	0.3±0.00^{c}	0.1±0.00^{c}	0.1±0.3^{b}	0.43±0.1^{a}	0.21±0.2^{a}	0.22±0.1^{a}
$F_{(0.05)}$	$_{(12.3)}$21.82	$_{(8.5)}$14.84	$_{(7.25.)}$13.09	$_{(6.5)}$11.34	$_{(3.32)}$06.11	$_{(0.82)}$43.99	--	--	--	--

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

Table V. Mortality of Acridid (Adults) population cultured in small jars under laboratory conditions (after treatment of *Aspergillus* oil formation).

Treatments	Days of observation (Mean ± SE)						
	1st	2nd	3rd	4th	5th	6th	7th
A. flavus	0.35±0.32^{b}	0.00±0.00^{d}	1.5±0.47^{a}	6.9±1.41^{a}	11.0±2.10^{a}	27.8±1.30^{a}	3.4±2.00^{a}
A. fumigatus	0.00±0.00^{c}	2.5±1.00^{a}	0.61±0.32^{c}	3.8±1.32^{b}	5.8±0.43^{b}	9.8±1.20^{c}	27.0±3.9^{b}
A. niger	1.42±0.31^{a}	1.00±0.58^{b}	1.00±0.43^{b}	4.5±0.53^{b}	4.9±1.02^{c}	11.42±1.30^{b}	22.8±1.90^{c}
Control	0.00±0.00^{c}	0.75±0.31^{c}	0.00±0.00^{d}	1.9±0.46^{c}	0.00±0.00^{d}	1.00±0.57^{d}	1.8±0.00^{d}
$F_{(0.05)}$	$_{(0.44)}$77.67	$_{(1.06)}$02.62	$_{(0.77)}$35.26	$_{(4.27)}$07.85	$_{(5.42)}$09.60	$_{(12.5)}$21.82	$_{(13.7)}$23.56

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

Table VI. Mortality of Acridid (Adult) population treated with conidial concentration in H_2O cultured maintained in the large cage.

Treatments	Days of observation (Mean ± SE)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. flavus	0.02±0.32^{b}	0.01±0.02^{b}	1.2±0.01^{a}	0.23±0.15^{b}	1.5±0.03^{a}	1.5±0.01^{a}	1.2±0.32^{b}	1.2±0.13^{c}	1.3±0.14^{b}	1.5±0.01^{a}
A. fumigatus	0.01±0.21^{c}	0.00±0.00^{c}	0.3±0.04^{c}	1.6±0.01^{a}	1.2±0.03^{b}	0.5±0.04^{c}	1.3±0.01^{a}	1.5±0.02^{a}	0.00±0.00^{c}	0.00±0.00^{b}
A. niger	0.2±0.23^{a}	0.1±0.12^{a}	0.1±0.15^{d}	0.23±0.1^{b}	0.5±0.7^{d}	0.2±0.15^{d}	0.01±0.2^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{b}
Control	0.01±0.02^{d}	0.00±0.00^{c}	0.5±0.23^{b}	0.02±0.1^{c}	0.6±0.22^{c}	0.7±0.23^{b}	0.00±0.00^{d}	1.3±0.01^{b}	1.4±0.01^{a}	1.5±0.04^{a}
$F_{(0.05)}$	$_{(0.06.)}$11.34	$_{(0.02)}$04.36	$_{(0.52)}$91.63	$_{(0.52)}$91.63	$_{(0.95)}$16.58	$_{(0.72)}$26.54	$_{(0.62)}$09.08	$_{(1.00)}$02.62	-------	-------

Note: Mean in the same column followed by the same letters is not significantly different from one another at 5% level of probability.

mating (aberrant mating practice), and oviposition behavior, typical behavioral responses to entomopathogenic infections (Mascarin *et al*., 2019), in infected insects. In addition, the spore dissemination through air, soil and water which we observed in this study confirms the earlier reports by Nawaz *et al*. (2024) investigations done by Ali *et al*. (2014) which provides some evidence of such a natural epizootic due to their apparent environmental adaptability. The choice of *Aspergillus* spp. considerations of their cosmopolitan distribution, speed of sporulation, and ease in culture, form the basis of this study. The choice of *Aspergillus* spp. in this study was based on their cosmopolitan distribution, rapid sporulation, and ease of culture. Although less commonly used in commercial biocontrol products compared to *Metarhizium*, *Beauveria* and *Aspergillus* strains have shown promising virulence in tropical conditions (Khan *et al*., 2022). In Pakistan, especially Sindh, their integration into IPM remains understudied and holds potential for sustainable pest suppression. This study advocates the use of fungal biopesticides as alternatives to chemical insecticides, particularly in sensitive agricultural ecosystems. However, the development of safe, targeted formulations is critical to avoid unintended effects on pollinators and other beneficial arthropods (Batista *et al*., 2021). Additionally, efforts must be made to promote mass production of conidia, molecular profiling of fungal strains, and field validation in diverse agroecological zones to ensure their effectiveness and regulatory acceptance.

CONCLUSION

Present study recommends that where indigenous *Aspergillus* spp. strains can be used as potential bio-control agents against various economical important grasshopper species in Pakistan. The significant morphologies and elemental composition differences of fungal isolates were displayed in scanning electron microscope (SEM) at a good extent and these upright features were useful in species identification and support their structural integrity. It was also observed that all the assayed Aspergillus strains were pathogenic, especially when used at initial stages of nymph stages, which were causing instant death. *Aspergillus niger*, *A. flavus* and *A. fumigatus* were characterized by high virulence and the highest mortality occurred in the first part of days before and after treatment in the laboratory and semi-field conditions. Also, the infected insects had decreased feeding behavior and anomalies in reproduction, which suppressed the physiological effects of the fungi. The results highlight the possibility of using *Aspergillus* spp. as potential biopesticides in the biosecurity pest management (IPM) strategies particularly in areas with mid-scale ecological imbalances due to the overuse of chemical pesticides. Still, to apply their wider applicability, additional studies of formulation optimization, field validation, and the safety of non-target organisms should be carried out. This study is important in the creation of sustainable and environmentally friendly pest interventions in the agro eco-systems.

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Data availability

Data presented in this study will be available on a fair request to the corresponding author.

Generative AI or AI-assisted technology statement

The authors declare that no generative AI or AI-assisted technologies were used in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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