Naturally Occurring Entomopathogenic Fungi Infecting Stored Grain Insect Species in Punjab, Pakistan

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ABSTRACT. The occurrence of entomopathogenic fungi isolated from stored grain insect pests sampled from various geographical regions of Punjab, Pakistan, was investigated. In total, 25,720 insects from six different species were evaluated, and 195 isolates from 24 different fungal species were recovered. These included the Ascomycetes Beauveria bassiana sensu lato (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae), Metarhizium anisopliae sensu lato (Metschnikoff) Sorokin (Hypocreales: Clavicipitaceae), Purpureocillium lilacinum (Thorn) Samson (Hypocreales: Ophiocordycipitaceae), and Lecanicillium attenuatum (Zare and W. Gams) (Hypocreales: Clavicipitaceae). The cadavers of red flour beetle Tribolium castaneum (Herbst.) (Coleoptera: Tenebrionidae) were significantly infected with the fungi followed by rice weevil Sitophilus oryzae (L.) (Coleoptera: Curculionidae), lesser grain borer Rhizopertha dominica (F.) (Coleoptera: Bostrichidae), rusty grain borer Cryptolestes ferrugineus (Stephens) (Coleoptera: Curculionidae), and cowpea weevil Callosobruchus maculatus (F.) (Coleoptera: Bruchidae); however, the least were recovered from khapra beetle Trogoderma granarium (Everts) (Coleoptera: Dermestidae). The geographical attributes (altitude, longitude, and latitude) greatly influenced the occurrence of entomopathogenic fungi with highest number of isolates found from >400 (m) altitude, 33°–34’ N latitude, and 73°–74’ E longitude. The findings of the current surveys clearly indicated that the entomopathogenic fungi are widely distributed in the insect cadavers, which may later be used in successful Integrated Pest Management programs.

Key Words: occurrence, entomopathogenic fungi, stored grain insects, localities, virulence

Grain commodities are commonly infested by insect pests during storage (Haq et al. 2005). It is generally considered that 5–15% grain losses in different stored grain commodities occur as a result in insect pest infestations (Padin et al. 2002), which reduces the commercial value, quality of stored grain and seed viability. The most economically important stored grain and pest species include the red flour beetle Tribolium castaneum (Herbst.) (Coleoptera: Tenebrionidae), the lesser grain borer Rhizopertha dominica (F.) (Coleoptera: Bostrichidae), and the rice weevil Sitophilus oryzae (L.) (Coleoptera: Curculionidae) (Bello et al. 2001; Chambang et al. 2007; Michalaki et al. 2007). These pests are often managed using synthetic chemical insecticides applied to the grain store; however, excessive insecticide use can result in control failure following the evolution of insecticide resistance and has also raised concerns about human and environmental safety (Cox 2004). As an alternative to these insecticides, entomopathogenic fungi have received some attention for use in stored product Integrated Pest Management (IPM) (Gillespie 1988). The potential benefits of using entomopathogenic fungi include human safety, the development of an effective against S. oryzae and R. dominica on rice, wheat, and maize grains. High mortality rates of R. dominica were attributed to using a local isolate of B. bassiana on stored wheat under laboratory conditions (Wakin et al. 2011, 2012). However, despite the clear potential of entomopathogenic fungi for use in IPM, little is known about the natural occurrence of fungal infections in populations of stored product pests, and this could be an impediment to the identification of the most effective candidate fungal strains. In the current study, we quantified the entomopathogenic fungal flora occurring on populations of grain insects in Pakistan in order to better understand the relationship, if any between fungal occurrence and geographical location.

Materials and Methods

Collection of Stored Grain Insects. Systematic surveys of grain storage facilities were carried out in different districts of Punjab (Pakistan). The relevant geographical attributes (Table 1) and the positions of the sites are given in Figure 1. In total, five to six grain storage facilities were sampled from 15 sites (Table 1). Each sample consisted of ~500 grain collected from each of five different points selected at random within the grain storage facility (i.e., a total of 2.5 kg grain was collected from each store). At the time of sample collection, any insects with abnormal movement or showing lethargic activity in the immediate vicinity of the subsample were also collected and put into sterile plastic vials. The grain samples were put into zip lock bags and were brought back to the laboratory; however, the vials containing insects were kept in a cooler maintained at 10°C before reaching the laboratory. The grain samples were sieved to separate any insects present from the...
Table 1. Sampling sites with geographical characteristics for the isolation of fungi from insect cadavers

| Sites         | Altitude (m) | Latitude     | Longitude   |
|---------------|--------------|--------------|-------------|
| Bahawalpur    | 109          | 29° 24′ N    | 71° 40′ E   |
| Lodhran       | 111          | 29° 53′ N    | 71° 63′ E   |
| Basti Maluk   | 119          | 29° 51′ N    | 71° 32′ E   |
| Multan        | 124          | 30° 20′ N    | 71° 48′ E   |
| Khanewal      | 128          | 30° 17′ N    | 71° 55′ E   |
| Shorot        | 126          | 30° 95′ N    | 72° 08′ E   |
| Jhang         | 158          | 31° 27′ N    | 72° 31′ E   |
| Faisalabad    | 184          | 31° 30′ N    | 73° 05′ E   |
| Sargodha      | 193          | 32° 10′ N    | 72° 40′ E   |
| Sheikhupura   | 213          | 31° 71′ N    | 73° 97′ E   |
| Lahore        | 210          | 31° 35′ N    | 74° 20′ E   |
| Gujranwala    | 223          | 32° 10′ N    | 74° 12′ E   |
| Ghakhar       | 224          | 32° 30′ N    | 74° 15′ E   |
| Jhelum        | 220          | 32° 55′ N    | 73° 43′ E   |
| Rawalpindi    | 497          | 33° 58′ N    | 73° 08′ E   |

Results

Occurrence of Fungi. In total, 195 insect cadavers were separated out of 25,720 insects collected during the survey of different grain storage facilities (Table 3). The major fungal genera isolated from the insect cadavers were Aspergillus sp. (22.6% occurrence), followed by Fusarium sp. (15.9%), Alternaria sp. (10.8%), Rhizopus sp. (8.2%), Penicillium sp. (6.7%); however, the least was Mucor sp. (1%) (Table 2). These fungal species were isolated from different insects as 68 from T. castaneum, 41 from S. oryzae, 26 from R. dominica, 21 each from C. ferrugineus and C. maculatus, and 18 from the khapra beetle T. granarium (Everts) (Coleoptera: Dermestidae). The fungus were distributed in all the collection sites surveyed (Table 3). The highest frequency (23.1%) of fungi was recorded from the various storage facilities in Rawalpindi district, followed by 12.8% from Ghakhar, 12.3% from Jhelum, and 8.7% both from Sargodha and Faisalabad; however, the lowest fungi was occurred (0.5%) in Lodhran, Punjab province, Pakistan.

Entomopathogenic Fungi. The entomopathogenic fungi were widely distributed in the cadavers collected from the grain-storage facilities located in various districts. There were four entomopathogenic fungal genera found from a total of 25,720 insects. The fungus B. bassiana was the most dominantly occurring species (3.6%) isolated from the cadavers compared with other fungi. On the other hand, the fungus M. anisopliae was the next important distributed with 2.1% occurrence. The distribution of fungus Purpureocillium lilacinum was minimal with 1.5% followed by Lecanicillium attenuatum (1.03%). In the present study, the geography of collection sites influenced the distribution of entomopathogenic fungi.

Among the four genera of entomopathogenic fungi, B. bassiana (43.8%) ranked at the top followed by M. anisopliae (25%), P. illacium (18.8), and L. attenuatum (12.5%) (Table 4). T. castanaeum was the most contaminated (0.3%) insect species with all types of mycoflora followed by S. oryzae (0.2%), R. dominica (0.1%), C. ferrugineus, and C. maculatus (0.1%) occurrence (Table 2). Among the entomopathogenic fungi, B. bassiana was the most frequent (5 isolates out of 195) isolated from T. castaneum; however, the least occurred were P. illacium and L. attenuatum (1 isolate of each out of 195). The geographical parameters were also studied, which influenced the occurrence of entomopathogenic fungi, in insects. The geographical coordinates (latitude, altitude, and longitude) of all the localities have influenced the distribution of entomopathogenic fungi as highest number of isolates were found from >400 altitude, 33°–34° N latitude, and 73°–74° E longitude (Fig. 2).
Virulence Test. The virulence (percentage mortality at 7-d post inoculation) of the recovered isolates from insect cadavers was quantified against larvae of *T. castaneum*, and the mortality was significantly \( P < 0.05 \) different among the isolates (Table 4). The mortality range from the lowest to the highest of *L. attenuatum* was (48.2–71.9%) with 59.8% mortality, followed by *P. lilacinum* (62.5–77.0%) with 67.8% mortality, and *M. anisopliae* (71.2–84.7%) with 76.9% mortality, and *B. bassiana* proved to be more virulent (76.4–92.7%) which gave 85.8% mortality (Table 4).

Discussion
Entomopathogenic fungi consist of a diverse group of species (Chandler et al. 1997) and have been recorded from all major taxa of...
arthropods (Roberts and Humber 1981). The key to successfully exploiting their potential as biological control agents lies in comprehensive knowledge of their ecology and life history (Fisher et al. 2011). There is a wide range of literature available about the natural occurrence of entomopathogenic fungi in the soil (Klingen et al. 2002; Shapiro-Ilan et al. 2003; Meyling et al. 2009; Batalla-Carrera et al. 2013; Wakil et al. 2013). However, to our knowledge, there have been few previous reports on the natural distribution of entomopathogenic fungi from stored grains insect pests from geographically distinct storage facilities.

Our findings clearly indicated the diversity in the occurrence of microbiota in storage insect pests. Along with entomopathogenic fungi, there were some other fungal genera recorded comprising of opportunistic pathogen and secondary colonizers. The present findings can be compared with a few available reports about the distribution of entomopathogenic fungi in storage structures: Odour et al. (2000) conducted the survey of 124 farms of maize in 12 districts of Kenya and found association of B. bassiana with S. zeamais, Tribolium sp., and Carpophilus sp. Thakur and Sandhu (2010) isolated 246 entomopathogenic fungal isolates, which belong to seven species of the fungi from Lepidopteran, Coleopteran, and Dipteran host insects. Hatting et al. (1999) collected eight species of entomopathogenic fungi during the survey of South Africa, which includes six Entomophthorales and two Hyphomycetes that infected and killed the aphid hosts. Similar to our

**Fig. 2.** Effect of geographical attributes (a, altitude; b, latitude; c, longitude) on the occurrence of entomopathogenic fungi from the insect cadavers from storage facilities of Punjab, Pakistan. The variables are categorized in groups indicated in the respective legend of the plots.
Approximately 3,400 insects were collected in three different surveys. Entomophthora muscae, E. aphidis, Entomophthora sp., B. bassiana, and other entomopathogenic fungal strains were pathogenic, significant variations were recorded among isolates of different genera and even within the same genera. In support of this statement, Alston et al. (2005) and Gindin et al. (2009) reported the differences in the larval mortality of Plum curculio, Conotrachelus nenuphar (Herbst) (Coleoptera: Curculionidae), and Lesser mealworm, Alphitobius diaperinus (Panzer) (Coleoptera: Tenebrionidae), by the application of entomopathogenic fungi. Different strains of Beauveria and Metarhizium are known to vary in virulence and other pathogenicity-related characteristics (Zimmermann 2007a,b; Anderson et al. 2011) are inconformity with our results. The present findings are in line with those of Aemprapa (2007) as he screened 7 isolates of Metarhizium sp., 12 isolates of Beauveria sp., and 1 isolate of Hirsutella citriformis against oriental fruit fly (Bactrocera dorsalis) (Hendel Diptera: Tephritidae). The mortality rate of the fruit fly was from 2 to 68%; however, Beauveria sp. (isolate 6,241) killed 50% of the fruit flies. Similarly, Er et al. (2008) tested the pathogenicity of eight entomopathogenic fungi (P. farinosus, P. fumosoroseus, B. bassiana, Lecanicillium lecanii, and M. anisopliae) against Coccinella septempunctata L. (Coleoptera: Coccinellidae). The mortality rate after 8 d varied between 27 and 51%, and there were statistically significant differences among the effects of the tested fungi. The pathogenicity of B. bassiana, I. fumosorosea, and M. anisopliae was confirmed against Leptoglossus occidentalis (Heidemann) (Heteroptera: Coreidae) and in the laboratory LC50 values were highest for I. fumosorosea and lowest for M. anisopliae (Barta 2010). In the previous study conducted by Italian authors (Rumine and Barzanti 2008), the B. bassiana virulence was proved against western conifer seed bug, L. occidentalis, adults under laboratory conditions. The five entomopathogenic fungal isolates (C. obscurus 79, C. obscurus 79-3, C. obscurus E68, and C. thomoneoides and Basidiobolus ranarum) were used against aphids Aphis fabae (Scopoli) and Metopeurus fuscoviride (Stroyan) (Hemiptera: Aphididae) and all fungal isolates tested were virulent (Halimona and Jankevica 2011). In another study, Sookar et al. (2008) tested 14 isolates of M. anisopliae, P. fumosoroseus, and B. bassiana against Bactrocera zonata (Saunders) (Diptera: Tephritidae) and B. cucurbitae and reported all were pathogenic. In our study, B. bassiana isolates caused highest mortality followed by M. anisopliae; this could be due to the use of native isolates as these isolates might be better adapted or prepared to infect a particular host that cohabit in the same location (Batalla-Carrena et al. 2013).

The entomopathogenic fungi are distributed in the insect species from the grain storage facilities; however, their presence was confined to the localities with relatively cooler climatic conditions. These fungi have great virulence against the stored grain insect species because of their pathogenicity against T. castaneum larvae. Further research is required for the exploration of indigenous isolates of these fungi so that they may play key role for the environment friendly and safer management of stored grain insect pests.

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