



Article Entomopathogenic Fungi in the Soils of China and Their Bioactivity against Striped Flea Beetles *Phyllotreta striolata*

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Abstract: The present research aims to explore the occurrence and diversity of entomopathogenic fungi (EPF) in cultivated and uncultivated lands from different provinces of China and to search for EPF against *Phyllotreta striolata*. In this study, first, the EPF biodiversity from the soil of four provinces (Hunan, Hubei, Henan and Hebei) was surveyed. There were 302 fungal isolates obtained from 226 soil samples collected from croplands (114), arbor (79), grasslands (97) and fallow land (12); 188 EPF isolates were identified as 11 genera. The data indicate that Hubei Province has the greatest EPF diversity, with a Shannon Evenness Index (SHEI) value of 0.88. Here, the grassland, arbor and cropland had an EPF diversity with SHEI values of 0.81, 0.86 and 0.76, respectively, while the fallow land had the highest SHEI value of 1.00, which suggests that cultivation by humans affected the count and richness of soil fungi: the less human activity, the more kinds of fungi found. Finally, the pathogenicity of 47 fungal strains against the adult *P. striolata* was determined. *Isaria javanica* (IsjaHN3002) had the highest mortality. In conclusion, this study reports the EPF distribution and biodiversity in the soil from four provinces in China, showing that the amount and type of fungi in the soil varied by region and vegetation and that soil was one of the resources for acquiring EPF. The potential of *I. javanica* as a biocontrol must be studied further.

Keywords: Isaria javanica; pathogenicity

1. Introduction

Entomopathogenic fungi (EPFs) are ubiquitous in nature. Biological plant protection with EPFs plays a key role in sustainable pest management programs [1]. In addition to absorbing nutrients for their own growth, some EPFs can control insect populations at low levels for long periods [2]. Fungi-based insecticides have great potential as a form of pest control [3]. Not only are EPFs are harmless to human beings, animals and crops, but they also have the advantages of long-term validity, non-resistance, no residue, no pollution, no damage to natural enemies, high epidemic potential and ease of production [4,5]. Therefore, using EPFs to control agricultural and forestry pests has become a new trend in pest control. EPFs are the largest group of insect-pathogenic microorganisms. According to incomplete statistics, about 100 genera and 1000 species of EPFs have been recorded around the world [6], and more than 40 genera and more than 400 species have been found in China [7], including *Beauveria*, *Metarhizium*, *Penicillium* and *Fusarium*. *Beauveria bassiana* and *Metarhizium anisopliae* have been extensively developed as mycoinsecticides [8]. These species are naturally present in agricultural soils, but the spore numbers in nature are often too low to result in the effective control of pest population outbreaks [9].

Through in-depth studies on the physiology, ecology and molecular biology of EPFs, the effect of applying EPFs to control insects has been significantly improved. Under the premise that pests generally develop resistance, more and more attention has been paid to sustainable development and pollution-free pest management, and researchers



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). prefer the development and utilization of EPFs [10]. Some fungi have a unique method of infection (they can infect pests through the main body wall), which cannot be replicated by other microbial insecticides. The process of EPFs infecting insects mainly includes host recognition, mechanical destruction, toxin secretion and metabolism interference. The combined effect of various factors leads to the death of the host insects [8]. The host species of EPFs are highly specific, and the host spectrum and virulence of different strains are also quite different. Therefore, the isolation and identification of more strains will help us to enrich the resources of EPF and provide more materials for the development of biological control pesticides using EPF [11].

Phyllotreta striolata (Coleoptera: Chrysomelidae) is a prominent pest of *Brassicaceae*, *Solanaceae*, *Cucurbitaceae* and *Leguminosae* vegetables [12–15]. Brassicaceae are important crops in south China [16]. Their management is based on synthetic chemical pesticides, leading to insect resistance [17,18]. Few registered varieties of biopesticides can meet the needs of green prevention and control. EPFs represent the most promising candidates in the integrated pest management (IPM) program approach [19].

Popular EPFs, such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Purpureocillium lilac-inum* and *Isaria* (=*Cordyceps*) *javanica*, have been developed as mycopesticides to control agricultural, forest and disease vector pests such as locusts, grubs, aphids, whiteflies, moths, mosquitoes and phytopathogenic nematodes [20,21]. It was found that *B. bassiana* and *M anisopliae* can infect the larvae and adults of *P. striolata* [22,23], but this research is still at the laboratory stage. Because most EPFs are soil-dwelling microbes, investigating soil fungi will be beneficial for exploring new species of EPF resources [24–26].

The Hebei, Henan, Hubei and Hunan provinces have complex and diverse landforms, with a variety of plateaus, mountains, hills, basins and plains, as well as a large latitude span in the Yellow River and Yangtze River basins, which have sufficient water and diverse climate types and are suitable for farming. They are the main agricultural production areas in China and have rich agricultural ecological landscapes. However, the distributions of soil EPFs in these regions are not clear. Therefore, this research aims to investigate the distribution and abundance of EPFs in different soil habits of these Chinese provinces. Moreover, the impacts of human activities and changes in the environment on EPFs are analyzed and discussed. The study of EPFs in the soil of the four areas is beneficial for the exploration of new strains to enrich the diversity of EPFs and for mining highly pathogenic strains.

2. Materials and Methods

2.1. Soil Sample Collection

The soil samples were collected from different sites (cropland, fallow land, arbor and grassland). The longitude and latitude of each site were recorded by ICEGPS 100C (Shenzhen, China). From each site, approximately 200 g of soil (10~15 cm depth) from three points was collected, mixed and stored in a plastic bag at 4 °C until further use. In total, 226 samples were collected from these sites (Table A1, Appendix A).

2.2. Isolation of Fungi from the Soil Samples

The method from our previous work was used to isolate fungal strains from the soil samples [27]. Soil suspensions of 0.02 g/mL were prepared with 0.1% Tween-80 solution; then, 0.1 mL of the suspension was inoculated onto a selective medium (PDA, 0.2 g/L cycloheximide, 0.2 g/L chloramphenicol and 0.013 g/L Bengal red) and cultured at 25 ± 1 °C. When the fungi grew out, a single colony was transferred onto the PDA plate and cultured at 25 ± 1 °C, purified and cultured until a new colony was formed [28].

2.3. Identification of Fungal Species and Analysis of Genetic Homology

The identification of fungal isolates was based on the morphological characteristics and similarity of the rDNA-ITS sequences. DNA extraction kits (DP3112, Bio-Teke, Beijing) were used to extract the total DNA from fungal isolates. The primers ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the ITS region on a T100TM Thermal Cycler (BIO-RAD, Hercules, CA, USA) via a standard PCR cycling protocol (94 °C for 3 min, 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min for 33 cycles, then 72 °C for 10 min). The obtained ITS rDNA sequences were submitted to GenBank and compared with similar sequences through the BLAST tool of NCBI. The phylogenetic trees of the fungi were constructed by MEGA X via the statistical method of maximum likelihood, a bootstrap test of 500 replications and the Jukes–Cantor model [29]. The fungal strains are listed in Table 1.

Table 1.	The	information	of	referred	fungal	strains.

Strain/Voucher	GenBank Accession Number	Geographic Origin	Reference
Acremonium exuviarum	NR_077167	Canada	[30]
Acrophialophora nainiana CBS 417.67	MK926894	China	Unpublished
Apiotrichum cacaoliposimilis ATCC 20505	NR_154671	USA	[31]
Arthrographis kalrae	AB506810	Japan	[32]
Arthropsis hispanica CBS 351.92T	HE965759	Spain	[33]
Aspergillus auricomus NRRL 391	NR_135388	ÛSA	[34]
Aspergillus crustosus NRRL 4988	NR_135366	USA	[34]
Aspergillus fumigatus ATCC 1022	NR_121481	USA	[30]
Aspergillus granulosus NRRL 1932	NR_135348	USA	[30]
Aspergillus niger ATCC 16888	NR_111348	USA	[30]
Aspergillus nomius NRRL 13137	NR_121218	USA	[30]
Aspergillus pseudodeflectus NRRL 6135	NR_135372	USA	[34]
Aspergillus sclerotiorum NRRL 415	NR_131294	USA	[34]
Aspergillus sydowii CBS 593.65	NR_131259	Japan	Unpublished
Aspergillus tanneri ATCC MYA-4905	NR_111840	UŜA	[30]
Aspergillus terreus var. subfloccosus CBS 117.37	NR_149331	Netherlands	[35]
Aspergillus udagawae CBM FA-0702	NR_137442	Japan	Unpublished
Auxarthron alboluteum UAMH 2846	NR_111137	Canada	[30]
Beauveria bassiana ARSEF 1564	NR_111594	USA	[30]
Beauveria bassiana ARSEF 8187	HQ444271	Canada	[30]
Beauveria bassiana CBS 465.70	MH859798	Netherlands	[36]
Beauveria bassiana CBS 110.25	MH854802	Sri Lanka	[36]
Beauveria pseudobassiana ARSEF 3405	NR_111598	USA	[30]
Chloridium aseptatum MFLU 11-1051	NR_158365	China	[37]
Chrysosporium lobatum CBS 666.78	NR_111087	Spain	[30]
Clonostachys grammicospora CBS 209.93	NR_137650	Netherlands	[38]
Clonostachys rosea f. catenulata CBS 154.27	NR_145021	Netherlands	[38]
Coniochaeta fasciculata CBS 205.38	NR_154770	Spain	Unpublished
Cordyceps cateniannulata CBS 152.83	NR_111169	Thailand	[30]
Cunninghamella elegans CBS 160.28	NR_154747	China	[39]
Cutaneotrichosporon dermatis CBS 2043	NR_130667	USA	Unpublished
Fusarium falciforme CBS 475.67	NR_164424	Netherlands	[40]
Fusarium keratoplasticum FRC S-2477	NR_130690	USA	[41]
Fusarium solani CBS 140079	NR_163531	Slovenia	[42]
Gongronella butleri CBS 102.44	JN206284	Netherlands	[43]
Gongronella butleri CBS 157.25	JN206607	Netherlands	[43]
Hawksworthiomyces taylorii CMW 20741	NR_155176	South Africa	[44]
Isaria cateniannulata ARSEF 6242	GU734760	Brazil	[45]
Isaria farinosa ARSEF 4029	HQ880828	USA	[46]
Isaria farinosa CBS 262.58	AY624179	Thailand	[47]
Isaria fumosorosea ARSEF 887	EU553334	Brazil	[48]
Isaria fumosorosea CBS 244.31	AY624182	Thailand	[47]
Isaria fumosorosea CBS 337.52	EF411219	Thailand	Unpublished
Isaria javanica CBS 134.22	DQ403723	USA	[49]

Strain/Voucher	GenBank Accession Number	Geographic Origin	Reference
Isaria javanica CHE-CNRCB 303/2	KM234213	Mexico	[50]
Lecanicillium coprophilum CGMCC 3.18986	NR_163303	China	[51]
Lecanicillium saksenae IMI 179841	NR_111102	United Kingdom	[30]
Malbranchea aurantiaca CBS 127.77	AB040704	Japan	[52]
Melanoctona tectonae MFLUCC 12-0389	NR_154194	Thailand	Unpublished
Metarhizium anisopliae CBS 657.67	MH859066	Netherlands	[36]
Metarhizium flavoviride CBS 218.56	MH857590	Czech	[36]
Metarhizium marquandii CBS 282.53	MH857200	Netherlands	[36]
Metarhizium marquandii CBS 182.27	NR_131994	Thailand	[47]
Metarhizium carneum CBS 239.32	NR_131993	Thailand	[47]
Metapochonia bulbillosa 38G272	EU999952	USA	[53]
Metapochonia bulbillosa CBS 145.70	AJ292397	UK	[54]
Metapochonia bulbillosa FKI-4395	AB709836	Japan	[55]
Mucor ellipsoideus ATCC MYA-4767	NR_111683	USA	[55]
Nectria mauritiicola NHRC-FC048	AJ558115	Russia	Unpublished
Oidiodendron fuscum UAMH 8511	NR_111035	Canada	[30]
Paecilomyces formosus CBS 990.73B	NR_149329	Netherlands	[56]
Paecilomyces variotii CBS 338.51	FJ389930	Netherlands	[56]
Penicillium chrysogenum CBS 306.48	NR_077145	USA	[30]
Penicillium subrubescens CBS 132785	NR_111863	Netherlands	[30]
Penicillium rubens CBS 319.59	MH857874	Netherlands	[30]
Penicillium rubens CBS 129667	NR_111815	Netherlands	[30]
Penicillium guttulosum NRRL 907	NR_144820	USA	[57]
Penicillium citrinum NRRL 1841	NR_121224	USA	[30]
Penicillium mirabile CBS 624.72	JN899322	Netherlands	[58]
Phialophora livistonae CPC 19433	NR_111824	Netherlands	[30]
Purpureocillium lilacinum CBS 284.36	NR_111432	USA	[30]
Purpureocillium lavendulum FMR 10376	NR_111433	Spain	[30]
Simplicillium cylindrosporum JCM 18169	NR_111023	Japan	[30]
Simplicillium minatense JCM 18176	NR_111025	Japan	[30]
Talaromyces pinophilus CBS 631.66	NR_111691	Netherlands	[30]
Talaromyces purpureogenus CBS 286.36	NR_121529	Netherlands	[30]
Talaromyces trachyspermus CBS 373.48	NR_147425	Netherlands	[30]
Talaromyces variabilis CBS 385.48	NR_103670	Netherlands	[30]
Tolypocladium album CBS 869.73	NR_155018	Japan	Unpublished
Trichurus terrophilus CBS 368.53	LN850976	Spain	Unpublished

2.4. Evaluation of the Shannon Evenness Index

The biodiversity of fungi and EPFs in different soils was evaluated using the Shannon Evenness Index (SHEI). The SHEI was calculated via the formula SHEI = $-\sum_{i}^{s} (Pi)(\ln Pi)/\ln S$, where s is the total number of species in the sample, *i* is the total number of individuals in one species, *Pi* is the proportion of species in the sample, $\ln Pi$ is the value of the natural logarithm of *Pi* and *S* is the total number of species.

2.5. Bioassay of the Fungal Strains against P. striolata

The isolates of fungal species were subject to a bioassay against *P. striolata* based on the work of [27]. In summary, fungal conidia suspensions of 1.0×10^8 spores/mL were prepared with 0.02% Tween-80 solution. Spore suspension concentrations of 1.0×10^4 , 1.0×10^5 , 1.0×10^6 , 1.0×10^7 and 1.0×10^8 spores/mL were prepared by culturing with a light cycle of 12:12 at 25 °C for 7 days. The population of *P. striolata* was fed with radish lumps, which changed every day. Adults were paralyzed with carbon dioxide and dipped into the conidial suspension for 20 s. The pest populations were surveyed every 24 h after treatment. The 0.02% Tween-80 solution was used as a control group. The experiment was replicated thrice, and 20 adults were used for each treatment.

2.6. Scanning Electron Microscopy

The samples were placed in a 2 mL centrifuge tube, fixed with 2.5% glutaraldehyde overnight, washed with physiological saline and dehydrated using a graded series of ethanol; isoamyl acetate was replaced overnight. They were vacuum-dried, fixed onto the platform and then coated with platinum with an ion coater before being observed using a scanning electron microscope.

2.7. Statistical Analysis

Analyses of the bioassay data were carried out using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). The data were expressed as mean \pm SD and were subjected to one-way ANOVA, followed by Duncan's multiple range test (DMRT). Significant differences were accepted at *p* < 0.05.

3. Results

3.1. EPF Species Diversity in the Soils of China

In total, 302 fungal isolates were purified. Among these, 188 EPF isolates were identified as belonging to 11 genera according to the morphological and molecular analyses. Purpureocillium lavendulum, with 69 isolates, was the dominant species, and the congeneric species Purpureocillium lilacinum had only 13 isolates (Figure 1, Table A1). The genus Metarhizium had three species—M. anisopliae, M. marquandii and M. sp.—for which 49, 33 and 17 isolates, respectively, were obtained (Figure 1, Table A1). Penicillium had six species—Penicillium subrubescens, Penicillium guttulosum, Penicillium rubens, Penicillium chrysogenum, Penicillium citrinum and Penicillium mirabile—with 12, 2, 3, 1, 11 and 6 isolates found, respectively (Figure 2, Table A1). Aspergillus had 12 species (Figure 2, Table A1). Talaromyces had four species (Figure 3, Table A1), and both Beauveria and Isaria had three species each (Figure 3, Table A1). Both Lecanicillium and Simplicillium had four species each (Figure 4, Table A1). Fusarium, Coniochaeta and Clonostachys each had six species (Figure 4, Table A1). Other species with one to four isolates were identified as Tolypocladium album, Acremonium exuviarum, Acrophialophora nainiana, Nectria mauritiicola, Hawksworthiomyces taylorii, Chloridium aseptatum, Trichurus terrophilus, Chrysosporium lobatum, Arthropsis hispanica, Malbranchea aurantiaca, Auxarthron alboluteum, Arthrographis kalrae, Melanoctona tectonae, Phialophora livistonae, Xenopolyscytalum pinea, Oidiodendron fuscum, Cutaneotrichosporon dermatis, Apiotrichum cacaoliposimilis, Mucor ellipsoideus, Gongronella *butleri* and *Cunninghamella elegans*. (Figure 5, Table A1). The other 73 isolates have not been classified yet. Obviously, Purpureocillium lavendulum, M. anisopliae, M. marquandii, Purpureocillium lilacinum and B. bassiana were the most abundant EPF species.

3.2. Distribution of Soil EPF in Different Regions

There were different numbers and isolating rates of EPFs in different regions. Compared with the average fungal isolating rates of 83.70% and 61.92% in all fungi and EPFs, Henan had the highest rate of >90% (Table 2). However, the Shannon Evenness Index indicated that Hubei and Hunan were districts with the highest EPF biodiversity, while Hunan and Hebei had the EPF biodiversity with SHEI values of 0.87 and 0.88, respectively (Table 2).

3.3. The Biodiversity of Soil EPF in Different Environments

There were different numbers and isolating rates of EPF in Central China. Compared with the average fungal isolating rates of 87.42% and 61.16% for all fungi and EPFs, cropland samples had higher rates of >69% (Table 3). However, the SHEI indicated that cropland had the lowest EPF biodiversity, while fallow land samples had the most abundant EPF biodiversity (Table 3).



Figure 1. Phylogenetic tree of Purpureocillium spp. (A) and Metarhizium spp. (B) isolates.

3.4. The Pathogenicity of Fungal Isolates against P. striolata

Forty-seven isolates were subjected to a bioassay against *P. striolata*. The results indicate that *I. javanica* (IsjaHN3002) had the highest mortality, and *Aspergillus* spp., *Fusarium falciforme*, *Lecanicillium* spp., *Metarhizium* spp. and *Talaromyces* spp. all had obvious pathogenicity against *P. striolata* (Table 4).



Figure 2. Phylogenetic tree of *Penicillium* spp. (A) and *Aspergillus* spp. (B) isolates.



Figure 3. Phylogenetic tree of *Talaromyces* spp. (A) and *Beauveria/Isaria* (B) isolates.



Figure 4. Phylogenetic tree of *Lecanicillium/Simplicillium* spp. (**A**) and *Fusarium/Coniochaeta/Clonostachys* (**B**) isolates.



Figure 5. Phylogenetic tree of other isolates.

Desien	Soil Sample	Isolate Number		Isolation	Rate (%)	EPF	OHEL
Region	Numbers	Fungi	EPF	Fungi	EPF	Species	SHEI
Hunan	54	97	39	85.19	62.96	9	0.87
Hubei	50	58	24	80.00	40.00	8	0.88
Henan	63	73	83	98.41	90.48	7	0.84
Hebei	59	74	42	71.19	54.24	8	0.78
Total	226	302	188	83.70 *	61.92 *	11	-

Table 2. The fungi isolation and biodiversity of different regions.

* The mean isolation rate (%) of all regions. EPF: Entomopathogenic fungi; SHEI: Shannon evenness index.

Table 3. The fungi isolation and biodiversity of different samples.

Destan	Soil Sample	Isolate Number		Isolation	Rate (%)	EPF	CHEL
Region	Numbers	Fungi	EPF	Fungi	gi EPF	Species	SHEI
Arbor	64	79	45	79.69	59.38	9	0.86
Crop	85	114	83	84.71	69.41	9	0.76
Fallow land	9	12	6	100.00	55.56	6	1.00
Grass	68	97	54	85.29	60.29	9	0.81
Total	226	302	188	87.42 *	61.16 *	11	-

* The mean isolation rate (%) of all vegetation types sampled. EPF: Entomopathogenic fungi, SHEI: Shannon evenness index. Arbor: lands covered with arbor forests; cropland: farming lands planted with crops; fallow land: farming lands with no crops; grass: lands covered with grass.

Table 4. The pathogenicity of fungal isolates against adults of P. striolata.

Isolated Strain	Species	Adjusted Mortality (%)
ApcaHN2402	Apiotrichum cacaoliposimilis	12.96 ± 0.56
AsgrHB3004	Aspergillus granulosus	26.19 ± 0.24
AsnoHB27S02	Aspergillus nomius	21.05 ± 0.72
AsnoHN03S02	Aspergillus nomius	12.96 ± 0.57
AspsHB3002	Aspergillus pseudodeflectus	21.88 ± 0.85
AsscHN1502	Aspergillus sclerotiorum	7.55 ± 0.35
AssyHE06A03	Aspergillus sydowii	19.30 ± 0.38
AstaHN1501	Aspergillus tanneri	45.24 ± 0.39
AsteHN01S02	Aspergillus terreus	15.71 ± 0.73
AsudHE13C01	Aspergillus udagawae	16.00 ± 0.44
BebaHA22A02	Beauveria bassiana	10.70 ± 0.19
ChasHA15A02	Chloridium aseptatum	1.28 ± 0.51
CudeHN1202	Cutaneotrichosporon dermatis	2.83 ± 0.10
FufaHN1901	Fusarium falciforme	28.07 ± 0.68
IsjaHB2602	Isaria javanica	9.52 ± 0.30
IsjaHN3002	Isaria javanica	67.86 ± 0.61
LecoHE07B01	Lecanicillium coprophilum	18.18 ± 0.33
LesaHB28S05	Lecanicillium saksenae	26.32 ± 0.45
MeanHE15B01	Metarhizium anisopliae	23.56 ± 0.37
MeanHE20B01	Metarhizium anisopliae	19.62 ± 0.45
MemaHA24A01	Metarhizium marquandii	4.55 ± 0.42
MemaHN26S01	Metarhizium marquandii	22.81 ± 0.91
Mema sp. HN2501	Metarhizium marquandii	5.63 ± 0.41
MeteHN13S01	Melanoctona tectonae	15.00 ± 1.12
MuelHB24N03	Mucor ellipsoideus	15.00 ± 0.60
NemaHA14B01	Nectria mauritiicola	16.33 ± 0.30
OifuHA06B01	Oidiodendron fuscum	1.85 ± 0.19
PeciHA25A01	Penicillium citrinum	9.74 ± 0.29
PesuHN1002	Penicillium subrubescens	6.67 ± 0.27
PhliHN2702	Phialophora livistonae	16.33 ± 0.57

Isolated Strain	Species	Adjusted Mortality (%)
PulaHA08C01	Purpureocillium lavendulum	3.92 ± 0.30
SicyHE17A02	Simplicillium cylindrosporum	15.00 ± 0.27
SimiHE17C01	Simplicillium minatense	8.16 ± 0.23
TapiHB23G01	Talaromyces pinophilus	7.02 ± 0.16
TapiHB23S01	Talaromyces pinophilus	31.58 ± 0.32
TatrHE03C03	Talaromyces trachyspermus	11.11 ± 0.20
TatrHE14A01	Talaromyces trachyspermus	6.56 ± 0.44
TatrHE18B01	Talaromyces trachyspermus	8.33 ± 0.21
ToalHN15S03	Tolypocladium album	11.67 ± 0.65
HA13B02	_	2.27 ± 0.31
HA17C01	_	11.43 ± 0.18
HB3003	_	23.81 ± 0.34
HB3102	_	8.87 ± 0.69
HE07A01	_	14.81 ± 0.32
HN06Y05	_	14.81 ± 0.41
HN20Z01	_	7.41 ± 0.23
HN28J01	_	1.96 ± 0.22
Control	_	3.33 ± 0.45

Table 4. Cont.

Data in the table are mean values \pm standard error. For each test, 20 *P. striolata* samples were used in each treatment, and the concentration of the fungal spore suspension was 1.0×10^8 spores/mL. The experiment was repeated three times.

3.5. The Pathogenicity of I. javanica against P. striolata

According to the results shown in Table 5, the number of muscardine cadavers increased with the spore concentration. The lethal rate of 1.0×10^8 spores/mL spore suspension treatment group was as high as 80%. When the spore concentration was lower than 1.0×10^6 spores/mL, no hyphae were observed on the body wall of *P. striolata* in the first 3 days. There was no significant difference in the rate of zombies in the groups treated with spore suspensions at concentrations of 1.0×10^4 and 1.0×10^5 , 1.0×10^6 spores/mL in the first 3 days, but there was a significant difference in the rate of zombies in the group treated with spore suspensions with concentrations of 1.0×10^7 and 1.0×10^8 spores/mL in the first 3 days. After the seventh day, the differences among the treatment groups were revealed. Compared with other treatment groups, there was a significant difference in the lethal rate of the spore suspension with a concentration of 1.0×10^8 spores/mL.

Table 5. Pathogenicity of I. javanica in different concentrations against P. striolata.

Concentration	Accu	mulated Mortality	y (%)	Muscardine Cadaver Rate (%)			
(Spores/mL)	1 d	3 d	7 d	1 d	3 d	7 d	
СК	$3.33\pm2.36~\mathrm{c}$	$6.67\pm2.36~\mathrm{c}$	$10.00\pm4.08~\mathrm{d}$	0	0	0	
$1.0 imes 10^4$	$10.00\pm4.08~\mathrm{bc}$	$18.33\pm2.36b$	$40.00\pm4.08~\mathrm{c}$	0	0	$6.67\pm4.71~\mathrm{c}$	
$1.0 imes 10^5$	$15.00\pm0~ab$	$23.33\pm2.36b$	$41.67\pm2.36~\mathrm{c}$	0	0	$11.67\pm4.71~\mathrm{c}$	
$1.0 imes10^6$	$13.33\pm 6.23~\mathrm{abc}$	$20.00\pm4.08b$	$53.33\pm4.71~\mathrm{b}$	0	$1.67\pm2.36\mathrm{b}$	$21.67\pm6.23b$	
$1.0 imes 10^7$	$21.67\pm8.5~\mathrm{a}$	$33.33\pm6.23~\mathrm{a}$	$61.67\pm4.71~\mathrm{b}$	0	$3.33\pm2.36b$	$26.67\pm2.36b$	
$1.0 imes 10^8$	$23.33\pm2.36~\mathrm{a}$	$36.67\pm4.71~\mathrm{a}$	$80.00\pm4.08~\mathrm{a}$	0	$8.33\pm2.36~a$	$41.67\pm2.36~\mathrm{a}$	

Muscardine cadavers were dead insects that grew mycelium. Accumulated mortality includes all dead insects. The data are the mean \pm SE on different days after treatment. Different letters indicate a significant difference (p < 0.05) determined by DMRT.

3.6. Scanning Electron Microscopy Observations of Infection Process of I. javanica

The results showed that the attachment of conidia of *I. javanica* to different parts of the body surface was very different. After 2 h, the attachment of conidia was observed. No attachment of conidia was found on the head, abdomen, shard or other smooth surfaces. The conidia were mainly attached to the bristly areas and internodes such as the antennae,



foot joints, chest and chest feet. The most densely attached site was the intersegmental membrane of the chest feet, followed by the foot joints (Figure 6).

Figure 6. The attachment of conidia of *I. javanica* on the body surface after inoculation for 2 h. (A) Chest internode; (B) hind foot internode; (C) foot end.

After 12 h of inoculation, some conidia began to germinate, forming short germ tubes at the top. Twenty-four hours after infection, the top of the germ tube expanded to form an appressorium and continued in the direction of the intersegmental membrane, forming tendrils (Figure 7A–C) and looking for a suitable invasion site. The germ tube could also directly invade the body wall (Figure 7D). At 48 h, hyphae began to grow between the foot internode, and new conidiophores and conidia sprouted (Figure 8A). Next, 48–72 h after inoculation, the surface of the insect body was gradually covered by mycelia until it was completely covered (Figure 8B–D). Through stereoscopic observation, the mycelia were observed to grow from the body surface on the third day, and then the mycelium coverage increased day by day (Figure 9), while the control group never experienced mycelial growth.



Figure 7. SEM observations of the inoculation of *I. javanica* for 12–24 h. (**A**) Spores germinate to form a short dental canal; (**B**) apical expansion to form an adherent cell; (**C**) adnexal extension; (**D**) bud tube invades the body wall.



Figure 8. SEM observations of the inoculation of *I. javanica* for 48–72 h. (**A**) At 48 h, hyphae grew between the foot nodes, and new conidiophores and new conidia were formed. (**B**) At 60 h, conidia germination in vitro produced new hyphae covering the hindfoot. (**C**) At 72 h, the end of the foot was covered with mycelia. (**D**) At 72 h, the hind foot was covered with mycelia.



Figure 9. Mycelial growth of *Phyllotreta striolata* infected by *I. javanica.* (**A**) 3 d; (**B**) 4 d; (**C**) 5 d; (**D**) 6 d; (**E**) 7 d.

4. Discussion

This study surveyed the EPF distribution at a broad scale in China. ITS sequences are small and easy to analyze and have been widely used in the phylogenetic analysis of different fungal species, but their accuracy is controversial. Therefore, the identification of the fungal species in this study has some defects. Undoubtedly, our results initially provide a large amount of information about the soil fungi in these areas. Moreover, the results indicate that the soil environment strongly impacts the distribution of EPFs. Compared to arbor and non-cultivated land, the cropland samples had fewer EPFs. The isolation rate of EPFs was not high, which showed that soil fungi were not abundant in these areas and that the sampling and isolation methods also affected the isolation of fungi. The EPF diversity may be affected by the use of fungicides in croplands. China is a heavy consumer of pesticides, and a large number of broad-spectrum fungicides such as carbendazim, chlorothalonil and azoxystrobin, etc., are sprayed on croplands and probably inhibit fungi [59,60].

EPFs can parasitize insects and cause insect diseases, including some obligate parasitism that may not cause insect death but that can reduce the vitality of the host insects and weaken them [61] or affect insect spawning [62]; as such, when using EPFs, we can observe changes in the behavior of host insects [63,64]. Some studies have suggested that insects can actively identify fungi, with the target location being the cell wall of the fungi, while the fungi will take a series of measures to evade the host's defenses in the face of insect recognition [65]. Therefore, the invasion of host insects by EPF is a process of mutual influence and interaction [66]. As a result, the body surface of *P. striolata* may be able to recognize *I. javanica*, and the resistance and defense of *I. javanica* may also take measures to promote the germination of conidia in advance. In view of this fact, we can further explore what receptor binds the cell wall of conidia of *I. javanica* to produce signal molecules and promote spore germination, determining the factors promoting spore germination and improving pathogenicity.

Through scanning electron microscope observation, 12 h after infection with *I. javanica*, some conidia began to germinate, as shown in Figure 7. After 24 h of infection, only some scattered spores germinated. Because of the hard shell and dense structure on the body surface, the structure of the body wall varies greatly in different parts, and the outer skin has hydrophobic components. However, in tests of the bioactivity of different concentrations of spore suspensions against *P. striolata*, it was found that the spore suspension concentration of *I. javanica* had a stimulative effect on the production of zombies. This may be the QS phenomenon observed in *I. javanica*, which refers to a change in the physiological and biochemical characteristics of the microbial population in the process of its growth due to an increase in the population density, showing the characteristics of a small number of bacteria or a single bacterium. Cells use the QS mechanism to carry out cell-to-cell communication so that they can coordinate in a complex environment, and their "team combat ability" better ensures that the whole population survives. At present, the study of QSM is mainly focused on bacteria, and QSM has also been reported in related fungi [67]. In recent years, more reports have confirmed that fungi have QSM [68,69] and have QSM pheromones that are similar to the bacterial regulation of the physiological behavior of fungi [70–72]. However, in-depth studies of fungal QSM have not been carried out. Therefore, in the production of fungicidal insecticides using *I. javanica*, we can choose the appropriate formulation or use new production technology to help *I. javanica* survive in the form of sporangia, and it can also attach to the body surface after application to invade the body faster and improve its pathogenicity.

Several species have not been reported as EPF, namely *Aspergillus*, *Lecanicillium*, *Monascus*, *Talaromyces* and *Fusarium*. Their pathogenicity against *P. striolata* was discovered, and their potential for pest control deserves further research. Our experiment will provide new insight into the distribution characteristics of EPF and the conservation of their biodiversity.

5. Conclusions

In conclusion, 188 EPF isolates were identified from 226 soil samples, and the amount and types of fungi in the soil varied by region and vegetation type. *Metarhizium*, with 89 isolates, was recognized as the dominant EPF species, whereas *Purpureocillium* and *Beauveria* (respectively with 81 and 11 isolates) were the richer genera. Finally, it was first reported that *I. javanica* had pathogenicity against *P. striolata*, and we described its infection process.

Author Contributions: K.Z. and X.Z. completed most of the experiments, including the collection of the soil samples, the isolation and identification of the fungi strains and the bioassay and data analysis. Q.H. designed the experiments and collected partial soil samples. K.Z. and Q.W. wrote the article. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Publicly available datasets were analyzed in this study. These data can be found here: https://www.ncbi.nlm.nih.gov/nuccore/?term=OM372687:OM373035[accn], submission ID SUB9030162; accessed on 25 January 2022.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. The information of the soil samples collected and fungal isolates.

		Site		_	ConBank	
NO.	Address	Latitude and Longitude	Sample Environment	Isolate	Access No.	Species
HB01	Xianning, Hubei	29.267 N, 113.746 E	Fallow land	HB01Z01	_	
	0			HB01Z02	-	
				PeruHB01Z03	OM372687	Penicillium rubens
				HB01Z04	-	
			Crop	HB01G01	-	
			1	PesuHB01G02	OM372688	Penicillium subrubescens
HB02	Xianning, Hubei	29.568 N, 114.193 E	Crop	-	-	
	0		Arbor	HB02S01	-	
				MeanHB02S02	OM372689	Metarhizium anisopliae
			Grass	TapiHB0201	OM372690	Talaromyces pinophilus
				HB0202	-	
				PemiHB0203	OM372691	Penicillium mirabile
HB03	Daye, Hubei	29.973 N, 114.667 E	Crop	HB03Y01	-	
			1	AsteHB03Y02	OM372692	Aspergillus terreus
				HB03Y03	-	1 0
			Grass	HB0301	-	
HB04	Huanggang, Hubei	30.372 N, 115.161 E	Fallow land	TapiHB04Z01	OM372693	Talaromyces pinophilus
	00 0		Crop	MeanHB04F01	OM372694	Metarhizium anisopliae
			1	AsudHB04F02	OM372695	Aspergillus udagawae
				AsfuHB04F03	OM372696	Aspergillus fumigatus
HB05	Xinzhou, Hubei	30.863 N, 114.881 E	Crop	Mema sp. HB05N01	OM372697	Metarhizium marquandii
				TapiHB05N02	OM372698	Talaromyces pinophilus
			Grass	TapiHB0501	OM372699	Talaromyces pinophilus
HB06	Huanggang, Hubei	31.257 N, 115.056 E	Arbor	HB06S01	-	5 1 1
	00 0		Fallow land	PulaHB06Z01	OM372700	Purpureocillium lavendulum
			Grass	AsteHB0601	OM372701	Aspergillus terreus
I IDO7	Marker Their	20.007 NI 114 4(2 E	Cross	Mema sp.	OM272702	Matadizione manana dii
HD07	wunan, Hubei	30.887 IN, 114.462 E	Grass	HB0701	0101372702	ivietarnizium marquanaii
				HB0702	-	
				PemiHB0703	OM372703	Penicillium mirabile
			Arbor	AsnoHB07S01	OM372704	Aspergillus nomius
HB08	Xiaogan, Hubei	31.030 N, 113.938 E	Crop	-	-	
			Grass	TapiHB0801	OM372705	Talaromyces pinophilus
				HB0802	-	
				PeciHB803	OM372706	Penicillium citrinum
				IsjaHB0804	OM372707	Isaria javanica
HB09	Xiaogan, Hubei	31.325 N, 113.580 E	Fallow land	GobuHB0901	OM372708	Gongronella butleri
				HB0902	-	
	Suizhou, Hubei	31.665 N, 113.269 E	Arbor	-	-	
			Grass	-	-	
HB11	Xiangyang, Hubei	31.948 N, 112.929 E	Crop	HB11Y01	-	
				PemiHB11Q01	OM372709	Penicillium mirabile
				ClgrHB11Q02	OM372710	Clonostachys grammicospora
HB12	Xiangyang, Hubei	32.178 N, 112.211 E	Grass	HB12A01	-	
				MeanHB12A02	OM372711	Metarhizium anisopliae
			Arbor	-		
HB13	Xiangyang, Hubei	32.307 N, 111.614 E	Crop	AsteHB13F01 HB13F02	OM372712 _	Aspergillus terreus
			Arbor	PuliHB13S01	OM372713	Purpureocillium lilacinum

NO. Address Latifiade and Longitude Sample Environment Isolate Centlank Access No. Species HB14 Shiyan, Hubei 32.02 N, E111.00 E Grass Action PalatB1401 OM372714 Parparaccillian larendalum HB15 Shiyan, Hubei 32.02 N, 110.579 E FalatB1503 OM372716 Parparaccillian larendalum HB16 Shennongia, Hubei 31.623 N, 110.508 E Grass MonTB11601 OM372710 Parparaccillian larendalum HB17 Shennongia, Hubei 31.514 N, 110.338 E Grass ContaH11711 OM372720 Parparaccillian factorena HB18 Yichang, Hubei 31.514 N, 110.338 E Grass ColaH11711 OM372720 Apsequibilia foniquiato HB18 Yichang, Hubei 31.266 N, 109.889 E Grass HB190 - - Advertilian value and transfere Apsequibilian value and transfere HB20 Enshi, Hubei 30.615 N, 110.513 F Grass PeriatB200 OM37272 Acrematum factorena Apsequibilian value and transfere HB21 Yichang, Hubei 30.615 N, 110.513 F Grass			Site				
HB14 Shiyan, Hubei 32.502 N, E111.100 E Grass PulaHB1401 OM322714 Purparecillium lavendulum HB15 Shiyan, Hubei 32.020 N, 110.679 E Fallow land - - - Purparecillium lavendulum HB15 Shiyan, Hubei 31.823 N, 110.509 E Grass - - Purparecillium lavendulum HB16 Shennongjia, Hubei 31.823 N, 110.508 E Grass - MedmTB1601 OM322715 Purparecillium lavendulum HB17 Shennongjia, Hubei 31.514 N, 110.338 E Grass Grass H017721 Conschaft faccindat HB18 Yichang, Hubei 31.266 N, 110.686 E Grass HB1901 - - HB18 Yichang, Hubei 30.267 N, 110.377 E Grass HB1901 - HB20 Ershi, Hubei 30.556 N, 109.889 E Grass HB1901 - HB21 Yichang, Hubei 30.615 N, 110.513 E Grass MeanH120101 OM327279 Metarkizium anisofiaf HB21 Yichang, Hubei 30.635 N, 110.513 E Grass Mean Apil - Apergillio inrubos HB22 Yichang, Hubei 30.645 N, 110.513 E Grass Mean Apil - - HB22 Yichang, Hubei	NO.	Address	Latitude and Longitude	Sample Environment	Isolate	GenBank Access No.	Species
HB15 Shiyan, Hubei 32.020 N, 110.679 E Fallow land Grass HB10	HB14	Shiyan, Hubei	32.502 N, E111.100 E	Grass Arbor	PulaHB1401 HB14S01	OM372714	Purpureocillium lavendulum
HB16 Shennongjia, Hubei 31.823 N, 110.508 E Grass Grass MeanHB1601 MeanHB1601 OM372716 Purparecelline miceohlabum MeanhB1601 HB16 Shennongjia, Hubei 31.514 N, 110.338 E Grass MeanHB1601 OM372718 Conicoheta fascialata MeanhB16802 HB17 Shennongjia, Hubei 31.514 N, 110.338 E Grass ColaHB16501 OM372712 Arborsciellan HB18 Yichang, Hubei 31.266 N, 110.686 E Grass HB1801 - HB19 Enshi, Hubei 30.007 N, 110.377 E Grass HB1801 - HB20 Enshi, Hubei 30.556 N, 109.889 E Grass HB1801 - HB20 Enshi, Hubei 30.615 N, 110.513 E Grass HB2002 OM372725 MearhEizum antesplate recellance HB21 Yichang, Hubei 30.615 N, 110.513 E Grass HB2002 - HB20301 OM372728 Perpriciblian relation HB21 Yichang, Hubei 30.668 N, 111.517 E Grass HB20501 - - HB22 Yichang, Hubei 30.691 N, 112.285 E <td>HB15</td> <td>Shiyan, Hubei</td> <td>32.020 N, 110.679 E</td> <td>Fallow land Grass</td> <td>– HB1501</td> <td>_</td> <td></td>	HB15	Shiyan, Hubei	32.020 N, 110.679 E	Fallow land Grass	– HB1501	_	
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HB18 HB19Yichang, Hubei31.266 N, 110.686 E GrassGrassHB1801-HB19Enshi, Hubei30.007 N, 110.377 EGrassHB1901-HB1902AccexAB1903OM372225Mctarbizium anisophizeHB20Enshi, Hubei30.556 N, 109.889 EGrassPeruHB2001OM372726Peruicillium rubensHB20Enshi, Hubei30.556 N, 109.889 EGrassPeruHB2001OM372727Accemanium cubensArborHB20501HB21Yichang, Hubei30.615 N, 110.513 EGrassMema sp. PulaHB2102OM372730Purpuracellium tacendulum Arbor-HB21Yichang, Hubei30.615 N, 110.513 EGrassMema sp. CropNd372731Arboringellius furningellius Arbori-HB22Yichang, Hubei30.658 N, 111.028 EFallow land CropTapiHB22701HB22Yichang, Hubei30.688 N, 111.517 ECropPeciHB23Y01HB24Jingmen, Hubei30.904 N, 112.185 EArborAsteHB23Y01OM372738Aspergillus furnigetus TapiHB23X01OM372738Aspergillus furnigetus ArborAsteHB24001OM3727378Aspergillus furnigetus TapiHB23X01Aspergillus furnigetus AsteHB24N01OM3727378Aspergillus furnigetus HB24002AsteHB2401-HB25Jingmen, Hubei30.991 N, 112.854 EArborHB2501HB26Xiaogan, Hubei30.868 N, 113.576 EArbor <td>HB17</td> <td>Shennongjia, Hubei</td> <td>31.514 N, 110.338 E</td> <td>Grass</td> <td>CofaHB1701 AsfuHB1702</td> <td>OM372721 OM372722</td> <td>Coniochaeta fasciculata Aspergillus fumigatus</td>	HB17	Shennongjia, Hubei	31.514 N, 110.338 E	Grass	CofaHB1701 AsfuHB1702	OM372721 OM372722	Coniochaeta fasciculata Aspergillus fumigatus
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MemaHB28S03 OM372753 Metarhizium marquandii					PulaHB28S02	OM372752	Purpureocillium lavendulum
					MemaHB28S03	OM372753	Metarhizium marquandii
MemaHB28S04 OM372754 Metarhizium marquandii					MemaHB28S04	OM372754	Metarhizium marquandii
LesaHB28S05 OM372755 Lecanicillium saksenae					LesaHB28S05	OM372755	Lecanicillium saksenae

		Site				
NO.	Address	Latitude and Longitude	Sample Environment	Isolate	GenBank Access No.	Species
HB29	Qianjiang, Hubei	30.373 N, 112.889 E	Crop	TatrHB29Y01 AcnaHB29Y02 HB29Y03	OM372756 OM372757	Talaromyces trachyspermus Acrophialophora nainiana
			Arbor	MeanHB29Y04 AsnoHB29S01	OM372758 OM372759	Metarhizium anisopliae Aspergillus nomius
				HB29S02 PuliHB29S03 HB29S04	– OM372760	Purpureocillium lilacinum
HB30	Jingzhou, Hubei	30.352 N, 112.338 E	Grass	ClroHB3001 AspsHB3002 HB3003	- OM372761 OM372762 -	Clonostachys rosea Aspergillus pseudodeflectus
			Crop	AsgrHB3004 –	OM372763	Aspergillus granulosus
HB31	Jingzhou, Hubei	30.043 N, 112.158 E	Grass	AsfuHB3101 HB3102	OM372764 _	Aspergillus fumigatus
			Crop	-		
HN01	Changsha, Hunan	28.203 N, 113.303 E	Crop	PulaHN01S01 AsteHN01S02	OM372765 OM372766	Purpureocillium lavendulum Aspergillus terreus
			Crop	AsfuHN01503 IsiaHN0102	OM372767 OM372768 OM372769	Aspergillus fumigatus Isaria javanica
HN02	Changde, Hunan	29.634 N, 111.840 E	Grass	MemaHN0201 PulaHN0202	OM372770 OM372771	Metarhizium marquandii Purmureocillium lavendulum
			Arbor	PeciHN02S01 AsteHN02S02	OM372772 OM372773	Penicillium citrinum Asperoillus terreus
HN03	Changde, Hunan	29.131 N, 111.706 E	Grass	HN0301 PesuHN0302	- OM372774	Penicillium subruhescens
			Arbor	MemaHN03S01 AspoHN03S02	OM372775 OM372776	Metarhizium marquandii Aspergillus nomius
HN04	Zhangjiajie, Hunan	29.424 N, 111.163 E	Orchard Grass	TapiHN04Y01	OM372777	Talaromyces pinophilus
HN05	Zhangjiajie, Hunan	29.348 N, 110.568 E	Arbor Grass	HN05S01	-	
HN06	Xiangxi, Hunan	29.034 N, 110.228 E	Arbor Grass	PulaHN06S01 TapiHN0601	OM372778 OM372779	Purpureocillium lavendulum Talaromyces pinophilus
			Crop	HN06Y01 PuliHN06Y02 ChasHN06Y03 HN06Y04 UN06Y05	OM372780 OM372781	Purpureocillium lilacinum Chloridium aseptatum
HN07	Xiangxi, Hunan	28.623 N, 109.547 E	Grass	HN06 Y05 HN0701	-	
			Arbor	Pula sp. HIN0702 PeciHN0703 PulaHN07S01	OM372782 OM372783 OM372784	Purpureocillium lavendulum Penicillium citrinum Purpureocillium lavendulum
			Alboi	AsscHN07S02	OM372784 OM372785	Aspergillus sclerotiorum
HN08	Huaihua, Hunan	26.963 N, 109.747 E	Grass	HN0801 PesuHN08S01	OM372786	Metarhizium marquandii Penicillium subrubescens
HN109	Huaihua Hunan	26 614 N 109 671 F	Grass	PulaHN08S02 HataHN0901	OM372788 OM372789	Purpureocillium lavendulum Hazukszvorthiomuces taulorii
11100	Tuantua, Tunan	20.01410, 107.071 L	01035	PuliHN0902 PesuHN0903	OM372790 OM372791	Purpureocillium lilacinum Penicillium subrubescens
			Arbor	PulaHN09S01 HN09S02	OM372792	Purpureocillium lavendulum
HN10	Yongzhou, Hunan	26.662 N, 111.493 E	Grass	HN1001 PesuHN1002 HN1003	_ OM372793 _	Penicillium subrubescens
HN11	Yongzhou, Hunan	26.063 N, 111.831 E	Arbor Arbor	HN10S01 FufaHN11S01	_ OM372794	Fusarium falciforme
HN12	Yongzhou, Hunan	25.528 N, 112.111 E	Fallow land Grass	– PulaHN1201 CudeHN1202	– OM372795 OM372796	Purpureocillium lavendulum Cutaneotrichosporon dermatis
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HN13	Chenzhou, Hunan	25.659 N, 112.729 E	Grass	TavaHN1301 BebaHN1302 HN1303	OM372797 OM372798 –	Talaromyces variabilis Beauveria bassiana
HN14	Chenzhou, Hunan	25.965 N, 113.042 E	Arbor Grass Arbor	MeteHN13S01 BebaHN1401 PesuHN14S01 PuliHN14S02	OM372799 OM372800 OM372801 OM372802	Melanoctona tectonae Beauveria bassiana Penicillium subrubescens Purpureocillium lilacinum
HN15	Hengyang, Hunan	26.426 N, 112.889 E	Grass	AstaHN1501 AsscHN1502	OM372803 OM372804 OM372805	Aspergillus tanneri Aspergillus sclerotiorum Aspergillus sclerotiorum
			Albor	Mema sp. HN15S02	OM372805 OM372806	Metarhizium marquandii
				ToalHN15S03 ToalHN15S04 FusoHN15S05	OM372807 OM372808 OM372809	Tolypocladium album Tolypocladium album Fusarium solani
HN16	Hengyang, Hunan	26.974 N, 112.425 E	Grass	Mema sp. HN1601	OM372810	Metarhizium marquandii
			Orchard	AsscHN16Z01	OM372811	Aspergillus sclerotiorum
HN17	Loudi, Hunan	27.440 N, 112.132 E	Grass Arbor	Asscriv(16202 PesuHN1701 MemaHN17S01 XepiHN17S02	OM372812 OM372813 OM372814 OM372815	Aspergittus scierottorum Penicillium subrubescens Metarhizium marquandii Xenopolyscytalum pinea
HN18	Loudi, Hunan	27.821 N, 111.763 E	Grass	MemaHN1801 HN1802	OM372816 OM372817	Metarhizium marquandii
HN19	Yiyang, Hunan	28.264 N, 111.712 E	Fallow land Arbor	PesuHN18Z01 MeanHN19S01 HN19S02 HN19S03	OM372818 OM372819	Penicillium subrubescens Metarhizium anisopliae
			Grass	FufaHN1901	OM372820	Fusarium falciforme
				Mema sp. HN1902	OM372821	Metarhizium marquandii
HN20	Yiyang, Hunan	28.525 N, 112.045 E	Grass Fallow land	BebaHN2001 PulaHN2002 PesuHN2003 HN20Z01	OM372822 OM372823 OM372824	Beauveria bassiana Purpureocillium lavendulum Penicillium subrubescens
				BebaHN20Z02	OM372825	Beauveria bassiana
				HN20Z03	OM372826	Metarhizium marquandii
HN21	Changsha, Hunan	28.222 N, 112.567 E	Crop	MeanHN21G01	OM372829	Metarhizium anisopliae
				HN21G02 TapiHN21G03	OM372830	Purpureocillium lavendulum Talaromyces ninorhilus
			Arbor	PulaHN21S01 Mema sp.	OM372827	Purpureocillium lavendulum
				HN21S02	011372828	ivieturnizium marquanaii
HN22	Xiangtan, Hunan	27.806 N, 112.511 E	Fallow land Arbor	HN22Z01	OM372832 -	Purpureocillium lavendulum
HN23	Xiangtan, Hunan	27.846 N, 113.017 E	Grass	Mema sp. HN2301	OM372833	Metarhizium marquandii
HN24	Hengyang, Hunan	27.229 N, 112.897 E	Arbor Grass	PulaHN2302 PeciHN2303 Pula sp. HN23S01 TavaHN2401 ApcaHN2402	OM372834 OM372835 OM372836 OM372837 OM372838	Purpureocillium lavendulum Penicillium citrinum Purpureocillium lavendulum Talaromyces variabilis Apiotrichum cacaoliposimilis
			Arbor	PesuHN2403 MeanHN24S01 HN24S02	OM372839 OM372840	Penicillium subrubescens Metarhizium anisopliae
HN25	Zhuzhou, Hunan	26.893 N, 113.374 E	Grass	Mema sp.	OM372841	Metarhizium marquandii
			Orchard	MeanHN25B01	OM372842	Metarhizium anisopliae

Isolate	GenBank Access No.	Species
MemaHN26S01	OM372843	Metarhizium marquandii
HN26S02	-	,
MeanHN26Z01	OM372844	Metarhizium anisopliae
PulaHN2701	OM372845	Purpureocillium lavendulum
PhliHN2702	OM372846	Phialophora livistonae
HN28J01	-	,
PuliHN28J02	OM372847	Purpureocillium lilacinum
PeruHN28J03	OM372848	Penicillium rubens
PulaHN29B01	OM372849	Purpureocillium lavendulum
MeanHN29B02	OM372850	Metarhizium anisopliae
ArhiHN2901	OM372851	Arthropsis hispanica
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HN26	Zhuzhou, Hunan	27.496 N, 113.486 E	Arbor	MemaHN26S01 HN26S02	OM372843 _	Metarhizium marquandii
			Fallow land	MeanHN26Z01	OM372844	Metarhizium anisopliae
HN27	Xiangxi, Hunan	27.914 N, 109.385 E	Grass	PulaHN2701	OM372845	Purpureocillium lavendulum
				PhliHN2702	OM372846	Phialophora livistonae
HN28	Huaihua, Hunan	27.896 N, 109.702 E	Orchard	HN28J01	-	D 111 111 1
				PuliHN28J02	OM372847	Purpureocillium lilacinum
LINDO	Uusibus Uunan	27.267 NL 100.025 E	Fallow land	PeruHIN28J03	OM372848	Penicilium rubens
1111129	Tiudillua, Tiullall	27.307 IN, 109.933 E	Tallow land	MeanHN29B02	OM372850	Metarhizium anisonliae
			Grass	ArhiHN2901	OM372851	Arthronsis hisnanica
HN30	Huaihua, Hunan	27.216 N. 110.420 E	Arbor	SimiHN3001	OM372852	Simplicillium minatense
				IsjaHN3002	OM372853	Isaria javanica
HN31	Shaoyang, Hunan	26.941 N, 110.638 E	Arbor	XepiHN3101	OM372854	Xenopolyscytalum pinea
HN32	Shaoyang, Hunan	26.322 N, 110.837 E	Grass	-	_	
HE01	Xingtai, Hebei	36.905 N, 114.559 E	Crop	MeanHE01A01	OM372855	Metarhizium anisopliae
			_	LecoHE01A02	OM372856	Lecanicillium coprophilum
			Grass	MeanHE01B01	OM372857	Metarhizium anisopliae
				NemaHE01B02	OM372858	Nectria mauritiicola
LIE02	Chillenhauser II-hei	2E 004 NL 112 7E9 E	A	LecoHE01B03	OM372859	Lecanicillium coprophilum
HE02	Shijiazhuang, Hebei	33.994 N, 113.738 E	Arbor	LeconE02A01 MoonUE02A02	OM372860	Matarhizium anicopliae
				HE02A02	0101372001	weiumizium unisopiiue
HE03	Baoding, Hebei	39.138 N. 115.536 E	Crop	LecoHE03A01	OM372862	Lecanicillium coprophilum
TILCO	buounig, meber	5).100 I () 110.000 E	crop	MemaHE03A02	OM372863	Metarhizium marauandii
			Grass	HE03B01	_	
				LecoHE03B02	OM372864	Lecanicillium coprophilum
			Poplar	ClgrHE03C01	OM372865	Clonostachys grammicospora
			-	MeanHE03C02	OM372866	Metarhizium anisopliae
				TatrHE03C03	OM372867	Talaromyces trachyspermus
HE04	Zhangjiakou, Hebei	39.273 N, 115.455 E	Poplar	AualHE04A01	OM372868	Auxarthron alboluteum
			C	ClgrHE04A02	OM372869	Clonostachys grammicospora
			Crop	L ocoHE04B02	- OM372870	Lecanicillium convonhilum
				Mema sp	0101372070	Ессинсинит сорторниит
				HE04B03	OM372871	Metarhizium marquandii
HE05	Zhangjiakou, Hebei	39.375 N, 114.866 E	Poplar	HE05A01	_	
	0.		-	LecoHE05A02	OM372872	Lecanicillium coprophilum
			Cron	Mema sp.	OM372873	Metarhizium marauandii
			erop	HE05B01	01/072070	
LIEOC	71	40 400 NT 114 020 E	Orchard	MeanHE05B02	OM372874	Metarhizium anisopliae
HE06	Zhangjiakou, Hebei	40.488 N, 114.838 E	Orchard	MeanHE06A01	OM372875	Metarnizium anisopiiae
				AssyHE06A02	OM372877	Asperaillus sudavii
				BebaHE06A04	OM372878	Beauveria bassiana
			6	Mema sp.	O) (070070	
			Crop	HE06B01	OM372879	Metarnizium marquanaii
				PuliHE06B02	OM372880	Purpureocillium lilacinum
HE07	Zhangjiakou, Hebei	41.267 N, 114.785 E	Crop	HE07A01	-	A
			C	AscrHE0/C01	OM372882	Aspergillus crustosus
			Grass	LecoHE07D01	OM372881	Lecanicillium coprophilum
HE08	Zhangjiakou Hebei	41 073 N 115 389 F	Grass	HE08A01	0101372883	сесинстит соргорнийт
TILUO	Zhangjiakou, Hebei	41.075 IN, 115.507 L	01455	HE08A02	_	
			Crop	AualHE08B01	OM372884	Auxarthron alboluteum
			1	AsfuHE08C01	OM372885	Aspergillus fumigatus
HE09	Chengde, Hebei	41.581 N, 116.023 E	Grass	MeanHE09A01	OM372886	Metarhizium anisopliae
	-			PulaHE09A02	OM372887	Purpureocillium lavendulum
			Elm	AsfuHE09B01	OM372888	Aspergillus fumigatus
			Crop	HE09C01	-	
11040			~	OituHE09C02	OM372889	Oidiodendron fuscum
HE10	Chengde, Hebei	42.001 N, 116.975 E	Grass	TapuHE10A01	OM372890	1alaromyces purpureogenus
			Стор	-	_	

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HE11	Chengde, Hebei	42.253 N, 117.143 E	Grass	PulaHE11A01 PesuHE11A02	OM372891 OM372892	Purpureocillium lavendulum Penicillium subrubescens
			Pine	CofaHE11C01	OM372893	Coniochaeta fasciculata
			Crop	AssyHE11D01	OM372894	Aspergillus sydowii
HE12	Chengde, Hebei	41.997 N, 117.655 E	Orchard	AsfuHE12A01	OM372895	Aspergillus fumigatus
			Grass	PulaHE12B01	OM372896	Purpureocillium lavendulum
			_	MeanHE12B02	OM372897	Metarhizium anisopliae
HE13	Chengde, Hebei	41.302 N, 118.038 E	Crop	HE13A01	-	
				MeanHE13B01 MeanHE12B02	OM372898	Metarhizium anisopliae
			Poplar	MeanFIE13D02	OM372899	Acportillus udagazinas
			ropiai	PulaHE13C02	OM372900	Purnureocillium lavendulum
				MeanHE13C03	OM372902	Metarhizium anisonliae
HE14	Chengde, Hebei	40.578 N, 117.704 E	Crop	TatrHE14A01	OM372903	Talaromyces trachyspermus
			Grass	_	_	
HE15	Tangshan, Hebei	40.108 N, 117.985 E	Crop	MeanHE15A01	OM372904	Metarhizium anisopliae
	0		1	Sicy sp. HE15A02	OM372905	Simplicillium cylindrosporum
			Arbor	MeanHE15B01	OM372906	Metarhizium anisopliae
				PemiHB15B02	OM372907	Penicillium mirabile
			Grass	SicyHE15C01	OM372908	Simplicillium cylindrosporum
				SimiHE15C02	OM372909	Simplicillium minatense
11047	m 1 171 ·	00 F04 NI 110 0/4 F	6	MeanHE15C03	OM372910	Metarhizium anisopliae
HE16	Tangshan, Hebei	39.584 N, 118.264 E	Grass	MeanHE16A01	OM372911	Metarhizium anisopliae
HE17	langshan, Hebei	39.490 N, 118.682 E	Grass	PeciHE17A01	OM372912	Penicillium citrinum
				DulaUE17A02	OM372913	Simplicillium cylinurosporum
			Orchard	Pula en HE17R03	OM372914	Purpureocillium lavendulum
			Officialu	SicvHF17B02	OM372916	Simplicillium culindrosporum
				BebaHE17B03	OM372917	Beauveria bassiana
			Poplar	SimiHE17C01	OM372918	Simplicillium minatense
			1	MemaHE17C02	OM372919	Metarhizium marquandii
HE18	Tangshan, Hebei	39.408 N, 117.954 E	Crop	PulaHE18A01	OM372920	Purpureocillium lavendulum
	0		1	TatrHE18B01	OM372921	Talaromyces trachyspermus
				Pula sp. HE18B02	OM372922	Purpureocillium lavendulum
				TrteHE18B03	OM372923	Trichurus terrophilus
			Poplar	MeanHE18C01	OM372924	Metarhizium anisopliae
HE19	Tianjin, Hebei	38.768 N, 117.184 E	Crop	MeanHE19A01	OM372925	Metarhizium anisopliae
				NemaHE19A02	OM372926	Nectria mauritiicola
			Orchard	Pula sp. HE19B01	OM372927	Purpureocillium lavendulum
11520	Canazhou Hohoi	29 151 N 115 740 E	Crop	DulaUE20A01	OM372928	Nieturnizium unisopiue
11E20	Cangzhou, meder	36.131 IN, 113.740 E	Clop	MemaHF20A01	OM372929	Metarhizium marauandii
			Grass	MeanHE20B01	OM372931	Metarhizium anisonliae
HE21	Hengshui, Hebei	37.719 N. 115.193 E	Crop	PemiHB21A01	OM372932	Penicillium mirabile
			Grass	PechHE21B01	OM372933	Penicillium chrysogenum
				Pula sp. HE21B02	OM372934	Purpureocillium lavendulum
				MemaHE21B03	OM372935	Metarhizium marquandii
HE22	Handan, Hebei	36.804 N, 115.193 E	Crop	ClroHE22A01	OM372936	Clonostachys rosea
				MemaHe22B01	OM372937	Metarhizium marquandii
HA01	Xinxiang, Henan	35.268 N, 113.974 E	Orchard	HA01A01	-	
			Crop	MemaHA01B01	OM372938	Metarhizium marquandii
114.00	T : T T	2E 004 NI 112 EE0 NI	Grass	- D-1-11402401	-	D
HA02	Linznou, Henan	55.994 N, 113.758 N	Crop	PulariA02A01	OM372939	Furpureocultum lavenaulum
			Arbon	MeanFIAU2BUL	OM272041	Ivieturnizium anisopiide
H 4 03	Linzhou Honon	35 928 N 112 655 E	Arbor	rulariA02C01	01015/2941	г игригеосшит шоепишит
HA04	Puyang Henan	36.090 N. 115 124 F	Crop	- TrteHA04A01	- OM372942	Trichurus terronhilus
111101	i uyung, i iciait	55.070 IN, 115.124 E	ciop	MemaHA04B01	OM372943	Metarhizium marquandii
				Pula en	-	
			Grass	HA04C01	OM372944	Purpureocillium lavendulum

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NO.	Address	Latitude and Longitude	Sample Environment	Isolate	Genbank Access No.	Species
HA05	Kaifeng, Henan	34.790 N, 114.485 E	Crop	-	-	
			Grass	PulaHA05B01 BebaHA05B02	OM372945 OM372946	Purpureocillium lavendulum Beauveria bassiana
			Crop	Mema sp. HA05C01	OM372947	Metarhizium marquandii
				TavaHA05C02	OM372948	Talaromyces variabilis
HA06	Kaifeng, Henan	34.895 N, 114.328 E	Crop	MemaHA06A01	OM372949	Metarhizium marquandii
				PulaHA06A02 HA06A03	OM372950	Purpureocillium lavendulum
			Poplar	OifuHA06B01 HA06B02	OM372951	Oidiodendron fuscum
			Grass	PulaHA06C01	OM372952	Purpureocillium lavendulum
				ChloHA06C02	OM372953	Chrysosporium lobatum
UA07	Zhanazhau Hanan	24 491 NI 112 020 E	Anhon	MeanHA06C03	OM372954	Metarhizium anisopliae
HA07	Znengznou, Henan	34.481 N, 113.030 E	Orchard	PeciHA07B01	OM372955 OM372956	Penicillium citrinum
			ortenard	Pula sp. HA07B02	OM372957	Purpureocillium lavendulum
			Crop	PeguHA07C01	OM372958	Penicillium guttulosum
HA08	Luoyang, Henan	34.555 N, 112.873 E	Crop	PulaHA08A01	OM372959	Purpureocillium lavendulum
				MemaHA08A02	OM372960	Metarhizium marquandii Dumuraacillium layan dulum
				MeanHA08B02	OM372962	Metarhizium anisonliae
				MemaHA08B03	OM372963	Metarhizium marquandii
				PulaHA08C01	OM372964	Purpureocillium lavendulum
			_	MemaHA08C02	OM372965	Metarhizium marquandii
HA09	Luoyang, Henan	34.768 N, 112.093 E	Crop	PulaHA09A01	OM372966	Purpureocillium lavendulum
			Poplar	MeanHA09A02 MomaHA09B01	OM372967 OM372968	Metarhizium anisopliae Metarhizium marauandii
			i opiai	MeanHA09B02	OM372969	Metarhizium anisonliae
				PulaHA09B03	OM372970	Purpureocillium lavendulum
HA10	Sanmenxia, Henan	34.797 N, 111.243 E	Crop	PulaHA10A01	OM372971	Purpureocillium lavendulum
				MeanHA10A02	OM372972	Metarhizium anisopliae
				Pula sp. HA10B01	OM372973	Purpureocillium lavendulum
				MemaHA10b02 MeanHA10B03	OM372974 OM372975	Metarhizium marquanan Metarhizium anisonliae
HA11	Sanmenxia, Henan	34.626 N, 110.914 E	Crop	MemaHA11A01	OM372976	Metarhizium marauandii
		•	erek.	MeanHA11A02	OM372977	Metarhizium anisopliae
				PulaHA11A03	OM372978	Purpureocillium lavendulum
			6	PulaHA11B01	OM372979	Purpureocillium lavendulum
			Grass	- Pula cp	-	
HA12	Nanyang, Henan	33.566 N, 111.185 E	Crop	HA12A01 Mema sp	OM372980	Purpureocillium lavendulum
				HA12A02	OM372981	Metarhizium marquandii
				BebaHA12B01	OM372982	Beauveria bassiana
				PuliHA12B02	OM372983	Purpureocillium lilacinum
			Cross	TrteHA12B03	OM372984	Trichurus terrophilus
HA13	Nanyang Henan	33 072 N 111 792 E	Crop		0101372983	Ригригеосппит шоепиинит _
11110	i tariyang) i tertari		erop	PuliHA13B01 HA13B02	OM372986	Purpureocillium lilacinum –
HA14	Nanyang, Henan	32.780 N, 112.707 E	Crop	PulaHA14A01	OM372987	Purpureocillium lavendulum
			_	NemaHA14B01	OM372988	Nectria mauritiicola
		00 401 NT 110 001 F	Grass	MemaHA14C01	OM372989	Metarhizium marquandii
HA15	Xinyang, Henan	32.401 N, 113.931 E	Crop	PulaHA15A01 ChasHA15A02	OM372990	Purpureocillium lavendulum Chloridium acontatum
			Grass	PulaHA15C01	OM372992	Purpureocillium lavendulum
HA16	Xinyang, Henan	32.338 N, 114.128 E	Crop	PulaHA16A01	OM372993	Purpureocillium lavendulum
			1	MaauHA16A02	OM372994	Malbranchea aurantiaca
			Grass	PulaHA16C01	OM372995	Purpureocillium lavendulum
HA17	Zhumadian, Henan	32.707 N, 114.109 E	Crop	PulaHA17A01	OM372996	Purpureocillium lavendulum
				PulaH 4 17 B01	OM37299/	ivieturnizium marquandii Purnureocillium lazendulum
			Grass	HA17C01	-	
				D 1 114 17000	()	

Site			_	Carport		
NO.	Address	Latitude and Longitude	Sample Environment	Isolate	Genbank Access No.	Species
HA18	Luohe, Henan	33.510 N, 113.980 E	Crop	PulaHA18A01 Pula sp. HA18B01 HA18B02	OM373000 OM373001	Purpureocillium lavendulum Purpureocillium lavendulum
			Grass	MeanHA18C01 PulaHA18C02 MemaHA18C03	OM373002 OM373003 OM373004	Metarhizium anisopliae Purpureocillium lavendulum Metarhizium marquandii
HA19	Pingdingshan, Henan	33.652 N, 113.370 E	Crop	MeanHA19A01	OM373005	Metarhizium anisopliae
			Grass	PulaHA19A02 MeanHA19B01 PulaHA19B02 MemaHA19C01	OM373006 OM373007 OM373008 OM373009	Purpureocillium lavendulum Metarhizium anisopliae Purpureocillium lavendulum Metarhizium marquandii
				Pula sp. HA19C02	OM373010	Purpureocillium lavendulum
HA20	Xuchang, Henan	34.052 N, 113.709 E	Crop	AssyHA19C03 MemaHA20A01 MemaHA20B01	OM373011 OM373012 OM373013	Aspergillus sydowii Metarhizium marquandii Metarhizium marauandii
			Grass	PulaHA20C01 MemaHA20C02 BebaHA20C03	OM373014 OM373015 OM373016	Purpureocillium lavendulum Metarhizium marquandii Beauveria bassiana
HA21	Zhoukou, Henan	33.978 N, 114.867 E	Crop	PulaHA21A01 MemaHA2102	OM373017 OM373018	Purpureocillium lavendulum Metarhizium marquandii
			Grass	Pula sp. HA21B01 PeciHA21B02	OM373019 OM373020	Purpureocillium lavendulum Penicillium citrinum
HA22	Shangqiu, Henan	34.350 N, 115.572 E	Crop	HA22A01 BebaHA22A02 PuliHA22B01	– OM373021 OM373022	Beauveria bassiana Purpureocillium lilacinum
			Grass	PuliHA22C01	OM373023	Purpureocillium lilacinum
HA23	Shangqiu, Henan	34.596 N, 115.109 E	Crop	TapuHA23A01 MemaHA23B01 HA23B02	OM373024 OM373025 _	Talaromyces purpureogenus Metarhizium marquandii
			Orchard	PemiHA23C01	OM373026	Penicillium mirabile
HA24	Kaifeng, Henan	34.771 N, 114.806 E	Crop	MemaHA24A01 PulaHA24A02 PuliHA24B01	OM373027 OM373028 OM373029	Metarhızıum marquandıı Purpureocillium lavendulum Purpureocillium lilacinum
HA25	Zhengzhou, Henan	34.838 N, 114.036 E	Crop	PeciHA25A01 MeanHA25A02	OM373030 OM373031	Penicillium citrinum Metarhizium anisopliae
			Grass	MeanHA25B01 PuliHA25B02	OM373032 OM373033	Metarhizium anisophae Purpureocillium lilacinum
			Crop Poplar	MeanHA25C01 MemaHA25D01 HA25D02	OM373034 OM373035 –	Metarhizium anisopliae Metarhizium marquandii

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