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Biodiversity and virulence characterization of entomopathogenic fungi isolated from soils in different regions of Nigeria

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Abstract

Background: Studying the diversity of the indigenous entomopathogenic fungi (EPF) is a prerequisite to effective insect pest control. The distribution and occurrence of EPF from the soil across Nigeria were evaluated. Three subsoil samples from three locations in each of the 11 states were used to isolate the fungi by the insect bait method. Three fungal rates, 1.0×10^2 , 5.0×10^1 and 7.5×10^1 spores/ml, were applied to the *Galleria* larvae.

Results: EPF occurred in 41.1% of the soil sampled. These fungi belong to five and an unidentified genus. High species diversity was observed in Abuja with 6 species, and a uniform diversity of 5 species in Gombe, Ibadan, Jos, Kano, Lagos, Nasarawa and Port Harcourt. The Shannon–Wiener index ranges from 2.48 to 1.84. Similarly, species evenness showed a distinct similarity across the 11 sampling states and ranges from 0.99 to 0.92. Simpson Diversity Index was found to be highest in Bauchi with 0.31 and lowest in Abuja with 0.19. The mortality of the *Galleria mellonella* (L.) larvae differed significantly ($P < 0.01$) with EPF species and time of exposure. There was also a strong ($P < 0.001$) relationship between larval mortality and conidial concentration within and among the species. The virulence of the isolated fungi on *Galleria mellonella* larvae based on LC_{50} differs with states (sampling site) even within the same species.

Conclusions: The present study uncovered the diversity and occurrence of EPF in soil across Nigeria. In addition, the results can be useful in selecting the suitable soils and best adapted EPF in a particular soil across the study area, or it could be the beginning of a molecular study.

Keywords: Entomopathogenic fungi, Biodiversity, Virulence, Soil conditions, Biocontrol, Nigeria

Background

Entomopathogenic fungi (EPF) are natural enemies of insect. They play a vital role in regulating the insect pest population. This group of fungi can be found in five out of the eight fungal phyla (Araújo and Hughes 2016). Important genera of EPF are *Metarhizium*, *Beauveria* and *Lecanicillium*. EPF lower insect density, thereby reducing their disease-producing activities and consequently

damage to crops (Sinha et al. 2016). The mode of actions deployed by EPF is unique from that of bacteria and viruses, it does not require ingestion, and rather they need contact action to invade their host directly through the cuticle.

Agricultural losses due to insect pests are a major threat to food security worldwide (FAO 2021). Crop losses in Nigeria and other African countries as a result of insect pests are estimated to be 49% of the expected total crop yield (Sharma et al. 2017), causing a loss of approximately US\$ 200 billion each year (Akinfenwa 2022).

Modern agricultural practices promote pests' development by the creation of a less stable natural ecosystem.

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Table 1 Soil sample collection states and geographical location

No	Sampling States	No. of soil samples	Geographical zone	Latitude and Longitude
1	Jos	9	North central	9.929973978 and 8.890041055
2	Nasarawa	9	North central	8.490423603 and 8.5200378
3	Abuja	9	North central	9.083333149 and 7.533328002
4	Bauchi	9	Northeast	11.68040977 and 10.19001339
5	Borno	9	Northeast	10.62042279 and 12.18999467
6	Gombe	9	Northeast	10.29044293 and 11.16995357
7	Kano	9	Northwest	11.99997683 and 8.5200378
8	Sokoto	9	Northwest	13.06001548 and 5.240031289
9	Port Harcourt	9	South–south	4.810002257 and 7.010000772
10	Ibadan	9	Southwest	7.970016092 and 3.590002806
11	Lagos	9	Southwest	6.443261653 and 3.391531071

Monocropping encouraged ideal food reserves which support pests' populations to develop higher densities than they would under a natural ecosystem (Sinha et al. 2016). Also, wide-spectrum synthetic chemical pesticides kill the natural enemies that regulate pests' populations. Despite the expensive nature of these chemical pesticides, their effect is temporal as the natural ability of insects allows them to develop resistance. Presently, over 500 insect species are resistant to one or more of these chemicals.

These among other issues stimulate the renowned interest is the use of EPF in the control of insect pests. Nearly, all insects order is prone to fungal disease. EPF have diversity in mode of nutrition from biotrophy to necrotrophy and a general tendency to overcome insect defence (Sharma et al. 2020). Most of the research in this field focuses on the pathogenicity of EPF against specific insect pests. However, pathogenicity may vary with genera and strains. Therefore, there is a need to explore the diversity and virulence in the native EPF as an alternative method of integrated pest management.

Methods

Description of soil sample collection sites and sample collection techniques

Ninety-nine soil samples were collected from five geopolitical zones in Nigeria, namely Northcentral, Northwest, Northeast, South–south and Southwest. This includes Kano and Sokoto states; Bauchi, Gombe and Borno states; Rivers states; and Oyo and Lagos states, respectively (Table 1). Three subsoil samples of approximately 100 g were collected from three locations in each of the states. Table 1 shows the soil sample collection sites and the geographical locations. At each site, rhizospheric soil was collected using a hand auger at a depth of 10–15 cm. The three subsoils from each

sampling site were mixed thoroughly to make a pooled sample. These samples were brought to the Ecology laboratory, Abubakar Tafawa Balewa University, and stored at 4 °C until further use.

Rearing of *Galleria mellonella*

Larvae of *G. mellonella* were reared at the Biological Science laboratory, Abubakar Tafawa Balewa University, according to the method described by Jorjão et al. (2018) with slight modification, using baby food cereal instead of wheat bran. Distilled water was heated to 80 °C, and honey, glycerol and bee wax were separately added while swirling vigorously. While still warm 50 ml of the baby cereal was added. 100 g of the medium above was placed in each 500 ml bottle, and 300 adult moths were added. The lid of the bottle was replaced by a wire mesh for ventilation, and the bottles were then wrapped with aluminium foil and incubated at 30 °C. After four weeks, 4th instar larvae became available and in additional 7–8 days, and the 5th instar were present.

Isolation of entomopathogenic fungi from soil

Thirty grams of soil sample was added to 300 ml of distilled water. The content was thoroughly mixed for half an hour using an electric stirrer. The supernatant was serially diluted up to 10^{-6} dilutions. 100 µl of the sample from 10^{-2} and 10^{-4} dilutions was inoculated and smeared on a Potatoes (PDA) dextrose agar containing chloramphenicol (600 mg/l) and dodine (100 µl/500 ml). The Petri dishes were incubated for 5–7 days at 25 °C. The fungal colonies identical to the targeted fungi were sub-cultured onto fresh PDA and identified microscopically using standard identification key. Pure isolates were stored in an agar slant and refrigerated at 4 °C.

Culture characterization

EPF isolates were culturally examined based on characteristics such as the pattern of growth, colony colour, shape and texture on PDA.

Morphological characterization

The isolated fungal cultures were identified microscopically using the slide culture technique (Fazeli-Dinan et al. 2016). A block of 1 cm² of potato dextrose agar was placed into a sterile petri dish containing a glass slide. A seven-day-old fungal culture was inoculated on the agar block and covered with a cover slide. The culture was incubated for five days at 28 °C. Once the fungal structure was observed, the cover slide was carefully removed and a drop of lactophenol cotton blue with lactic acid was added. The slide was viewed under a microscope, and images were captured. Morphological identification keys of Zare and Walter (2007) were used to identify the fungi. Each fungal species was identified based on conidia shape and size, conidiogenous cell and colony growth rate.

Fungal spores' suspension preparation

The spore suspensions of all isolates were prepared by growing the isolates on potato dextrose agar (PDA) for 14 days at 25 °C. The fungal spores were scraped using a sterile spatula and transferred into 10 ml of sterile distilled water containing 0.1% Tween 80. Mycelium was removed by filtering the suspensions with layers of cheesecloth. Spore concentrations of the filtrates were determined by enumerating the number of spores in 10 µl of each fungal suspension using Hemocytometer at 400 × magnification.

Estimation of entomopathogenic fungi diversity

The EPF diversity in the study area was determined using the ComEcoPac software version 1.0 (Drozd 2010). The diversity parameters evaluated include the Shannon–Wiener index (H'), Simpson's index (D), species abundance, species richness (R) and evenness of fungal communities (E_H). These diversity indices were measured according to the formula:

$$\text{Shannon Index } (H') = \sum_{i=1}^s P_i \ln P_i$$

$$\text{Simpson Index } (D) = \frac{1}{\sum_{i=1}^s P_i^2}$$

where p is the number (n/N) of individuals of specific species found (n) divided by the total number of

individuals found (N), Σ is the sum of the calculations, \ln is the natural log, and S is the number of species.

$$\text{Evenness } (E_H) = \frac{H'}{H_{\max}}$$

where H' is the calculated Shannon–Wiener diversity; $H_{\max} = \ln(s)$ species diversity under maximum equitability conditions.

Pathogenicity assay of selected isolates against *Galleria mellonella*

The pathogenicity of the major and consistent isolates was tested against *G. mellonella* larvae as described by Yakubu et al. (2018). Twelve larvae of 3rd to 4th instar were selected and placed in a Petri dish lined with moistened filter paper. Ten larvae were immersed in 2 ml of conidial suspension of different concentrations of 1.0×10^2 , 5.0×10^1 and 7.5×10^1 spores/ml. Each isolate was replicated four times. Control was maintained by dipping the larvae in 2 ml sterile distilled water. The treated larvae alone with the container were kept at 25 °C. The number of dead larvae was recorded at 24 h intervals for the following 10 days.

Data analysis

The spore production data were analysed using a one-way analysis of variance. The percentage mortality data were normalized using arcsine transformation (Khan et al. 2015) and subjected to analysis of variance using Minitab version 17 (Minitab Inc., USA). The significant difference between the means was determined by Tukey's test at $P < 0.05$. The LC_{50} and LC_{90} values were determined by probit analysis (Finney 1971).

Results

Biodiversity and morphological characterization of fungi

A total of 111 (41.1%) EPF species belonging to five and an unidentified genus were identified from 127 soil samples. Based on morphological features, *Metarhizium*, *Beauveria*, *Verticillium*, *Aspergillus*, *Fusarium* and others were identified (Fig. 1A).

The cultural characteristics of the *Metarhizium* isolates revealed greenish colour colonies on the top side, whereas the backs of the colonies were either brownish, white or yellowish-white. *Metarhizium* colonies had a round, thin to thick addressed texture and flat to slightly raise elevation (Fig. 1A).

Beauveria colonies are white to yellowish with a smooth powdery to cottony texture, round in shape and showed a dispersed pattern of growth (Fig. 1B). *Lecanicillium* colonies were whitish to pale yellow, pinkish-brown, red, green or yellow. The reverses of the colonies

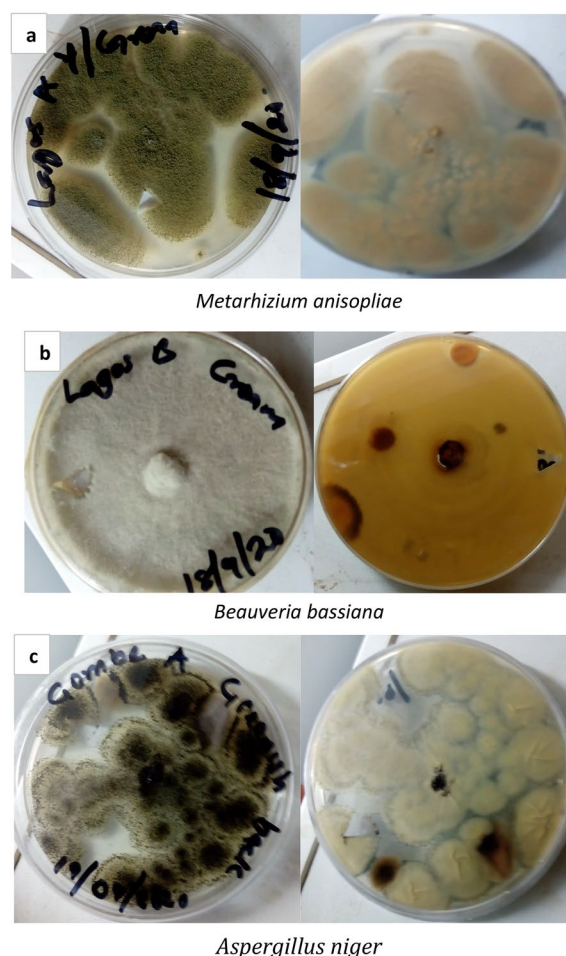


Fig. 1 Front and reverse photograph of some entomopathogenic fungal colonies on PDA with, **A** *Metarhizium anisopliae*, **B** *Beauveria bassiana*, **C** *Aspergillus niger*

were either colourless, yellow or reddish-brown. Conidiospores are well-differentiated and erect, vertically branch-bearing whorls of slender awl-shaped divergent phialides. *Aspergillus* colonies were initially white or bluish-green powdery becoming black or yellow-green powdery later with darkly pigmented conidia. The reverses of the colonies are pale yellowish. The surface of the colonies is suede-like consisting of a dense felt of conidiospores (Fig. 1C).

Distribution and occurrence of the entomopathogenic fungi

The distribution and occurrence of the EPF isolated from the 11 states across Nigeria are summarized in Table 2. Out of the 111 (41.1%) EPF isolated, *Aspergillus* was the highest in number with 22.5%, followed by *Beauveria* and an identified genus with (19.8%) each. *Lecanicillium* (18.5%), *Metarhizium* and *Fusarium* had relatively low numbers with (15.3) and (4.5%), respectively. The highest number of isolates was recorded in Gombe and Kano states with (12.6%) each, followed by Lagos, Nasarawa and Port Harcourt with (10.8%) each. Abuja (9.9%), Ibadan and Sokoto recorded (9.0%) each, and Bauchi (8.1%) and Jos recorded the lowest isolates with (6.3%).

Diversity indices

The variation pattern of the EPF isolated from the different sampling sites was evaluated, and species richness, species abundance, species diversity indices and evenness were determined as shown in Table 3. Species richness is the measure of different kinds of species in an area. The data obtained showed a relatively low species diversity in the 11 states: 6 species in Abuja, a uniform diversity of 5 species in Gombe, Ibadan, Jos, Kano, Lagos, Nasarawa

Table 2 Distribution of entomopathogenic, *Metarhizium*, *Beauveria*, *Aspergillus*, *Fusarium*, and unidentified species from different sampling sites

States	<i>Metarhizium</i>		<i>Beauveria</i>		<i>Lecanicillium</i>		<i>Aspergillus</i>		<i>Fusarium</i>		Others		Total	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Abuja	1	5.9	1	4.5	3	15	2	8	2	40	2	9.1	11	9.9
Bauchi	2	11.8	–	–	1	5	4	16	–	–	2	9.1	9	8.1
Gombe	3	17.6	3	13.6	2	10	3	12	–	–	3	13.6	14	12.6
Ibadan	–	–	2	9.1	3	15	1	4	3	60	1	4.5	10	9.0
Jos	1	5.9	2	9.1	1	5	2	8	–	–	1	4.5	7	6.3
Kano	3	17.6	3	13.6	1	5	4	16	–	–	3	13.6	14	12.6
Lagos	3	17.6	3	13.6	2	10	2	8	–	–	2	9.1	12	10.8
Nasarawa	2	11.8	3	13.6	2	10	3	12	–	–	2	9.1	12	10.8
Port Harcourt	2	11.8	3	13.6	3	15	1	4	–	–	3	13.6	12	10.8
Sokoto	–	–	2	9.1	2	10	3	12	–	–	3	13.6	10	9.0

Table 3 Species richness, abundance, Shannon index, evenness and Simpson's index of six indigenous entomopathogenic fungi isolated from the different geographical zones in Nigeria

	Abuja	Bauchi	Gombe	Ibadan	Jos	Kano	Lagos	Nasarawa	Port Harcourt	Sokoto
Species richness	6.00	4.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.00
Species abundance	11.00	9.00	14.00	10.00	7.00	14.00	12.00	12.00	12.00	10.00
Shannon–Wiener index	2.48	1.84	2.31	2.17	2.24	2.22	2.29	2.29	2.23	1.97
Evenness	0.96	0.92	0.99	0.93	0.96	0.95	0.99	0.99	0.96	0.99
Simpson's index	0.19	0.31	0.20	0.24	0.22	0.22	0.21	0.21	0.22	0.26

Table 4 F-values for three-way analysis of variance result on the differential effect of entomopathogenic fungi *Galleria mellonella* larvae

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Days	9	451,553	50,172.5	107.48	0.000
EPF	3	6177	2059.1	4.41	0.004
Conc	2	435	217.3	0.47	0.628
Days*EPF	27	9133	338.2	0.72	0.845
Days*Conc	18	1794	99.7	0.21	1.000
EPF*Conc	6	14,510	2418.4	5.18	0.000
Days*EPF*Conc	54	9266	171.6	0.37	1.000

and Port Harcourt, while Bauchi and Sokoto recorded the lowest species richness with 4 species each.

The results of species abundance revealed a higher number of species in Gombe and Kano with 14 species each, followed by Lagos, Nasarawa and Port Harcourt with 12 species, Abuja with 11 species, Ibadan and Sokoto with 10 species each, and the lowest abundance was observed in Bauchi and Jos with 9 and 7 species, respectively (Table 3).

Shannon–Wiener index is the most preferred diversity index, is a measure of randomness and was relatively the same across all the states. The Shannon index ranged from 2.48 to 1.84. Similarly, species evenness showed a distinct similarity across the 11 sampling states and ranged from 0.99 to 0.92. Simpson's Diversity Index measures the probability of two individuals randomly selected belonging to a species. Simpson index was found to be highest in Bauchi with 0.31 and lowest in Abuja with 0.19 but was relatively the same in the rest of the states (Table 3).

Pathogenicity of fungal isolates against *Galleria mellonella*

The analysis of variance of the effects of the EPF on *G. mellonella* larvae was significantly affected by days (time), EPF and EPF by concentration interaction (Table 4). The mortality of the *Galleria* larvae showed a progressive increase with an increased number of days, and mortality was significantly ($P < 0.001$) apparent from

the fifth day after inoculation (Fig. 2A). Mortality was significantly higher ($P < 0.01$) and relatively the same in *Metarhizium*-, *Lecanicillium*- and *Beauveria*-treated larvae than in *Aspergillus* treatment (Fig. 2B). At the same time, there was a significant ($P < 0.001$) EPF by concentration interaction, suggesting that larval mortality differed with concentrations between and among the different entomopathogenic fungi. Mortality was significantly higher in larvae treated with 5.0×10^1 spores/ml *Metarhizium* than in 7.5×10^1 spores/ml *Metarhizium*, 5.0×10^1 and 7.5×10^1 spores/ml *Aspergillus* and in 5.0×10^1 and 1.0×10^2 *Beauveria* treatments (Fig. 2C).

The virulence of the EPF conidia on *G. mellonella* larvae as expressed in terms of LC_{50} showed a significant variation among the isolates (Table 5). However, this variation differed with states (sampling site) even within the same isolates.

Metarhizium strain isolated in Bauchi had the lowest LC_{50} (0.551 spores/ml) which signified a highest virulence than those isolated from Kano (5.914 spores/ml) and Sokoto (1202.073 spores/ml). Relatively lower LC_{50} was also recorded in *Lecanicillium* (3.375 spores/ml) and *Aspergillus* species (7.391 spores/ml). Mean mortality values that did not share the same are significantly different

Discussion

In the present study, *Aspergillus niger* was found to have the highest rate of occurrence (22.25%), followed by *Beauveria* and an unidentified species (22.2% each). Ferial et al. (2019) observed *Aspergillus* species to be associated with a large number of insects. Here, *Lecanicillium* and *Metarhizium* recorded a moderate occurrence with 18.5 and 15.3%, respectively. The genus *Fusarium* recorded the lowest occurrence with 4.5%. This is in contrast with the findings of Bueno-Pallero et al. (2020) who observed that *Fusarium* had a frequent occurrence rate of 26%. Species in the genus *Fusarium* exhibited a high diversity of life strategies and types of associations with insects.

Fusarium is a weak virulent EPF, and it acts as a saprophyte on dead insects (Sharma and Marques 2018). The

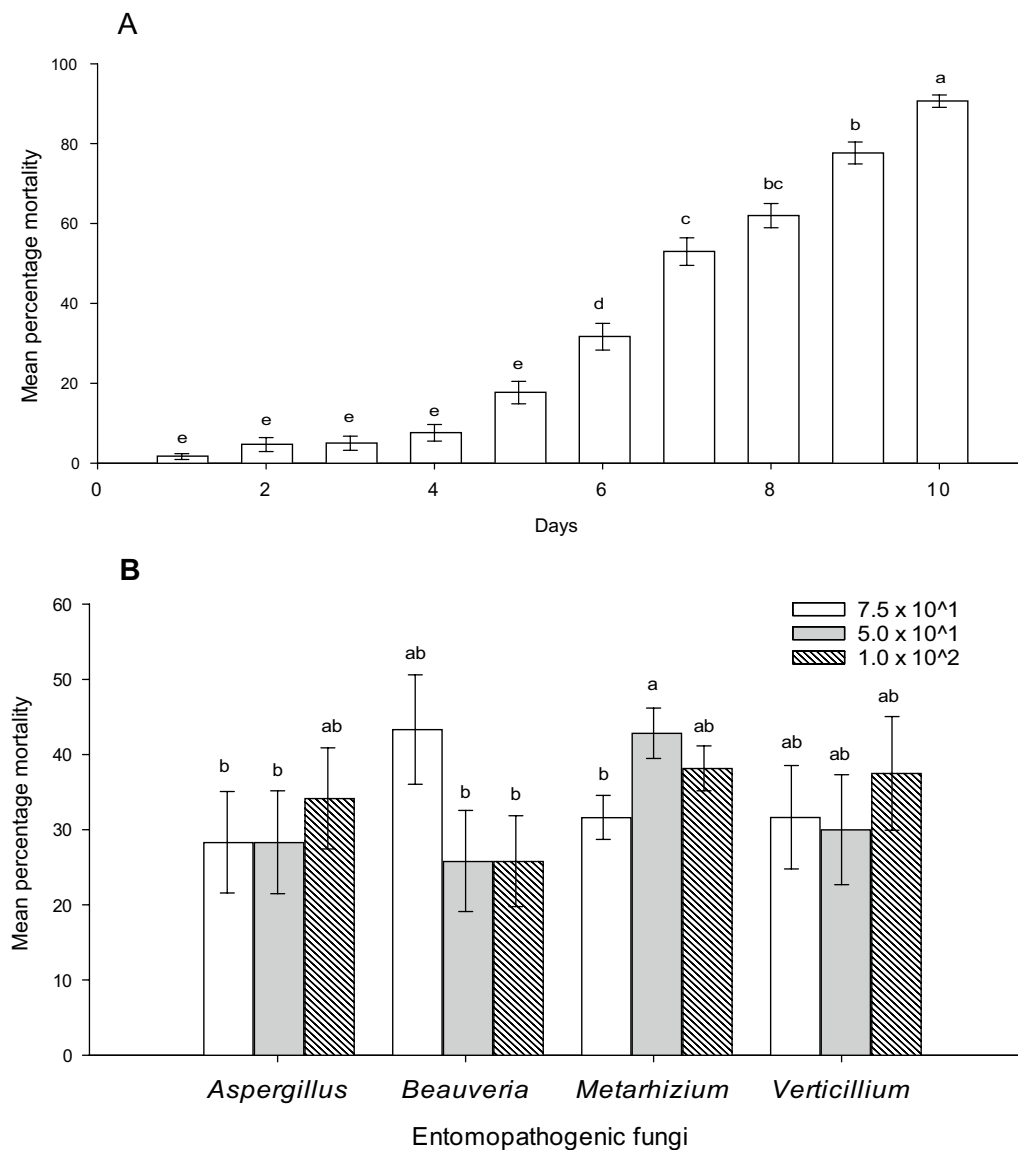


Fig. 2 Mean percentage larval mortality with **A** time after treatment, **B** entomopathogenic fungi and concentration. The differential effect of the entomopathogenic fungi on *Galleria mellonella* larvae. Data are mean ($n = 4$). Bar with different letters is significantly different, Tukey HSDs ($P < 0.05$)

diversity indices of the isolated species showed a uniform diversity of five species identified in Gombe, Ibadan, Jos, Kano, Lagos Nasarawa, Kano and Port Harcourt. Bauchi and Sokoto recorded the lowest species richness. However, the highest species abundance was observed in Gombe and Kano, while the lowest was seen in Bauchi and Jos. The distribution of EPF was affected by the geographical location. This may be due to differences in temperature, humidity and soil conditions (e.g. moisture and organic matter). This suggestion is supported by the finding of Abdelghany (2015) who observed that increased organic matter aided infection by EPF. In addition, higher

humidity is required by EPF for germination (Sharma et al. 2020). The Shannon–Wiener index, which is a measure of randomness, was found to be generally the same across all the states. While a high Simpson Diversity Index was recorded in Bauchi, it was found to be fairly the same in the rest of the states.

All the isolated EPF showed pathogenicity to *Galleria mellonella* larvae. The larval mortality rate was significantly affected by the time and the EPF genera. The mortality showed a progressive increase with the increased number of days which was apparent from five days after inoculation. This finding is in agreement with

Table 5 The percentage mortality and LC₅₀ of the entomopathogenic fungal isolates on *Galleria mellonella* larvae

States	EPF species	Mortality (%)	LC ₅₀ (spores/ml)	X2	Slope	Intercept
Bauchi	<i>Metarhizium</i>	42.33b	0.551	0.719	− 1.684	4.560
Kano	<i>Metarhizium</i>	32.92bc	5.914	0.923	0.484	4.625
Nasarawa	<i>A. species</i>	30.28bc	7.391	0.935	0.512	4.554
Port Harcourt	<i>Lecanicillium</i>	33.05a	3.375	0.951	0.656	4.653
Sokoto	<i>Beauveria</i>	31.67bc	190.001	0.674	0.179	4.537
Sokoto	<i>Metarhizium</i>	3.722bc	1202.073	0.952	0.101	4.688

abc: The letters signifies that mean mortality values that did not share the same are significantly different

the finding of Yakubu et al. (2018) who reported that mortality induced by *Metarhizium anisopliae* on *Aedes aegypti* mosquito larvae increased progressively with time following treatment. *Metarhizium*, *Lecanicillium* and *Beauveria* were not different, although they induced a significantly higher larval mortality than *Aspergillus*-treated larvae. This study further demonstrated that mortality varied with EPF species and conidia concentration. This was evidenced by the strong EPF by concentration interaction, suggesting that larval mortality differed with concentrations between and among different EPF species. This finding agreed with the results of Erler and Ates (2015) who reported that larval mortality varies with the conidia concentration and time after application. Similarly, using single conidial concentration mortality of *Spodoptera littoralis* differed among *Beauveria bassiana*, *Metarhizium brunneum* and *Clonostachys rogersoniana* (Şahin and Yanar 2021).

The virulence characteristics of the isolated EPF on the *G. mellonella* larvae, as indicated in terms of LC₅₀, revealed a strong-intraspecies difference among the isolates. Three strains of *Metarhizium* isolated from different states differed in virulence against *G. mellonella* larvae. *Lecanicillium* showed higher virulence than some strains of *Metarhizium* and *Aspergillus* species.

Conclusions

Six genera and 111 species of EPF were identified in the present study in soil across Nigeria. The abundance of these strains was directly affected by geographical location physical which may be associated with the spatial variation in soil chemical and physical properties. These indigenous strains of EPF are a promising alternative to the chemical control of insect pests. In addition, the results can be useful in selecting the suitable soils and best adapted EPF in a particular soil across the study area, or it could be the beginning of a molecular study.

Abbreviations

ANOVA: Analysis of variance; cm: Centimetre; EPF: Entomopathogenic fungi; G.: *Galleria*; °C: Degree celsius; No.: Number; ml: Millilitre; PDA: Potato dextrose

agar; LC₅₀: Lowest concentration the causes 50% mortality; LC₉₀: Lowest concentration the causes 90% mortality; µg: Microgram; µl: Microliter; LSD: Least significant difference; mg: Milligram; L: Litre.

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Author contributions

The research work was conceptualized by MM and conducted by HU, FD, SI, HU and MY under the supervision AA. The manuscript was written by MY and edited by Professor FT. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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