Trop J Nat Prod Res, July 2023; 7(7):3509-3516 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Molecular Characterization of Fungal Rhizosphere Community of Soya Beans (*Glycine max* (L.) Merril) and Their I*n Vitro* Antagonistic Activity

Chiemeka N. Onaebi[1], Nneka V. Chiejina[1], Dandy A. Osibe[1], Faustina N. Ugwuja[2], Augusta C. Okoro[1], Christopher C. Onyeke[1], Angela N. Amujiri[1], Kingsley O. Omeje[3], Hyacinth C. Obayi[1], Emmanuel G. Njoku[1]

[1]*Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria*
[2]*Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria.*
[3]*Department of Biochemistry, University of Nigeria, Nsukka, Nigeria*

ARTICLE INFO

Article history:
Received 06 February 2023
Revised 17 April 2023
Accepted 08 June 2023
Published online 01 August 2023

ABSTRACT

Rhizosphere fungal community play a crucial role in the development and productivity of plants. Microscopic fungi have shown considerable promise for root pathogen control, which calls for knowledge of their colonization and distribution in the rhizosphere. Herein, studies on the fungal community associated with the soya bean rhizosphere and their *in vitro* antagonistic activity were studied. Soya bean seeds were planted and soils collected from the rhizospheric region at maturity for fungal isolation. Molecular characterisation verified the isolates' identification based on their macroscopic and microscopic characteristics. DNA were extracted from the fungi and PCR amplification of the ITS gene region were performed. Dual culture technique was used to screen for fungal antagonism. Eighteen fungi comprising of *Aspergillus niger* , *A. fumigatus* , *A. flavus* , *A. tamari*, *A. hortai* , *A. nidulans*, *A. arcoverdensis*, *Rhizopus delemar*, *Penicillium pinophilum*, *Paecilomyces lilacinus*, *Blastobotrys proliferans*, *Talaromyces pinophilus*, *Neosartorya fischeri*, *Botrytis cinerea*, *Mucor micheli*, *Fusarium oxysporum*, *Helminthosporium solani*, and *Trichoderma asperellum* were isolated from the rhizosphere of soya beans. The most predominant species in the plant rhizosphere was *A. niger* and the least abundant species was *A. fumigatus*. *T. asperellum* exhibited *in-vitro* antagonistic activity against all the isolates except *R. delemar*. These inhibitory effects ranged from 7.83 to 83.88% for the isolates tested. Interestingly, *B. cinerea* was found to be more susceptible to *T. asperellum* antagonism compared to other isolates tested. Thus, this study suggests that soya bean rhizosphere harbours diverse fungal communities that could be explored for preventing plant infections using biological means.

***Keywords*:** Antagonism, Biological control, Fungi, Rhizosphere, Soya beans

Introduction

The rhizosphere is a sophisticated micro-ecological region directly influenced by plant roots. It serves as hotspot for the interaction of a wide range of organisms with their physical environments, including bacteria, fungus, protozoa, and nematodes. These interactions support soil fertility, organic matter decomposition, nutrient cycling, plant feeding, soil structure, and pathogen suppression.[1-3] More so, the rhizosphere offers a unique nutritive environment to diverse microbes as the plant roots provide constant supply of nutrients.[4] Interestingly, the physicochemical properties of the soil are known to have influence on the fungal communities of the rhizosphere. The availability of water, pH, and organic matter content are some of the components that determine rhizosphere communities in the soil. Tension is generated within the leaf as water transpires across its surface, and this tension is eventually transmitted to the root system. This could change the rhizosphere's water potential and cause significant changes in the amount of water and solutes that are available to microorganisms. [5] By favoring those microorganisms with superior systems for absorbing water, this fluctuation in the amount of water available could potentially put pressure on the diversity and total biomass of microbes in the rhizosphere. [6]

In the rhizosphere, microbial interactions can range from advantageous symbiotic connections to harmful pathogenic interactions.[6] These pathogenic interactions in the rhizosphere may be useful in the suppression of normal growth and activity of plant pathogens in the soil. Soil borne plant pathogens cause a significant annual loss of numerous economically significant crops. Toxicological and environmental Concerns have caused a sharp decline in the supply of effective commercial inhibitory chemicals, fungicide use also runs the risk of fostering the development of new, pathogen-resistant strains.[7] Reports have shown that significant proportion of microorganisms found in the environment could profoundly affect the growth of some phytopathogens *in vitro*. For example, *Trichoderma* species have attracted a lot of attention as possible biological control agents for fungi that cause plant diseases.[8] Consequently, the use of biocontrol agents is gaining increasing interests as efficient alternative to fungicides made of chemicals are used to control soil-borne diseases. Competition for nutrients and space,[9] the secretion of chitinolytic enzymes,[10-11] mycoparasitism, and the creation of inhibitory chemicals have all been linked to the efficiency of biocontrol agents.[12] Additionally, the higher metabolic rates, anti-microbial metabolites, and physiological conformation of these fungi all contribute to their hostile behaviors.[13] If the antagonistic activities of fungal rhizosphere communities can promote the control among plant pathogens found in soil, the screening of rhizospheric microorganisms for their biocontrol abilities is of

*Corresponding author. E mail: chiemeka.onaebi@unn.edu.ng
Tel: 08037292947

Citation: Onaebi CN, Chiejina NV, Osibe DA, Ugwuja FN, Okoro AC, Onyeke CC, Amujiri AN, Omeje KO, Obayi HC, Njoku EG. Molecular Characterization of Fungal Rhizosphere Community of Soya Beans (*Glycine max* (L.) Merril) and Their *In Vitro* Antagonistic Activity. Trop J Nat Prod Res. 2023; 7(7):3509-3516 http://www.doi.org/10.26538/tjnpr/v7i7.33

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

biotechnological importance. Thus, the aims of this study were to characterize the fungi of rhizosphere community connected with soy beans and screen for their potential inhibitory effects on isolated fungi during their interactions *in vitro*. This may enhance the knowledge of agricultural management systems that may improve the production of certain agricultural important crops using soy bean as a model.

Materials and Methods

The site and planting materials

The research was conducted in June, 2018 at the New Botanic Garden, Department of Plant Science and Biotechnology (PSB), University of Nigeria, Nsukka, Enugu State, Nigeria. It is located on Latitude $7^0 23'$ East, Longitude: $6^0 52'$ North, and 447.2 m above sea level.[14] The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, provided the soya bean seeds for this study. Seventy-five centimeter (75 cm) between rows and ten centimeters (10 cm) between stands within a row served as the spacing for sowing seeds, which were sown 2 to 5 cm deep each hole.

Soil sampling and chemical analysis

Rhizosphere soil samples were collected from the soil around the roots of the plant into a sterile polythene bags using the method described by Oyeyiola.[16] The soil physicochemical attributes of the study site were determined before planting of the seeds and at full maturity of the plants. The components of the soil physicochemical attributes examined in our study were soil texture, water holding capacity, moisture content, amount of organic matter content and pH. Chemical analyses of the soil properties were performed at the Department of Soil Science, UNN using standard laboratory methods as previously described by Vibhute.[17]

Estimation of rhizosphere fungal populations

Fungi were isolated, using dilution plate method, from the rhizosphere soil.[18] In brief, 10 g of the rhizosphere soil was poured into a conical flask containing 1000 ml of distilled water and shaken vehemently. Five-fold serial dilutions (1:100, 1:1000, 1:10000, 1:100000 and 1:1000000) were made from the stock solution as described by Shivanna and Vasanthakumari.[19] Thereafter, 0.1 ml aliquots of 1:10000 dilution of the inoculum were spread on Petri plates containing 39 g/L PDA medium (Difco, Sparks, MD, USA) with 30 mg/l of streptomycin. The plates were incubated at a laboratory temperature of $28\pm2\ ^0$C for 3-4 days. The percentage occurrence of the isolated fungi was calculated using the method of Sule and Oyeyiola.[4] Each species' percentage occurrence was counted, expressed as a percentage after being divided by all isolated species. The formula used to perform the computation is shown below:

(%) frequency of occurrence

$$= \frac{\text{No of occurrence of a species}}{\text{Total no of occurrence of all the species isolated}} \times \frac{100}{1}$$

Microscopy

The macro- and microscopic identification of the fungal isolates were performed using the method described by Ogunleye and Ayansola.[20] Colonies formed on the PDA medium were observed for their conidia colours, reverse plate colours, and texture. For microscopic identification, colonies were placed on clean slides, teased with lactophenol cotton blue, covered with clean cover slips, and viewed under a microscope. Using common mycological identification textbooks, the identity of fungi was verified.[21-24] Using a Motic camera microscope, photomicrographs of each fungal isolate were taken.

DNA extraction and PCR protocol

DNA extraction, sequencing, and BLAST (Basic Local Alignment Search Tool) were performed at IITA, Ibadan following the protocol of Srideepthi.[25] DNA extraction was performed and for PCR amplification, internal transcribed spacer (ITS) region of the rRNA gene was used for characterization of fungi. ITS universal primer set which flank the ITS 1, 5.8S and ITS 2 regions were also used. The nucleotide sequence of the ITS 1 primer is 5' TCC GTA GGT GAA CCT GCG G 3' and that of the ITS 2 is 5' TCC TCC GCT TAT TGA TAT GC 3'. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR program consisting of an initial denaturation at 94 °C for 5 min; followed by a 30 cycles consisting of denaturing at 94 °C for 30 sec elongation at, annealing of primer at 55°C and 72°C for 1 min 30 sec; and a final termination at 72°C for 10 min. The PCR products were chilled at 4°C.

Screening for antagonism between the fungal isolates

The screening for antagonism between the fungal isolates was done using dual culture technique as described by Devi and Chhetry.[26] The PDA plates were inoculated in opposite direction with actively developing 3 mm discs of test fungi and antagonistic isolates. The plates were kept incubating at 28 ± 2^0C temperature in the laboratory. Three replicates for each isolate and a control without inoculation of the antagonists were set up. After 6 days of incubation, the pathogen's percentage growth inhibition was determined with the formula below.

$$\% \text{ growth inhibition} = \frac{r1 - r2}{r1} \times \frac{100}{1}$$

Where, r1 = test pathogen's radial development in the control plate
r2 = test pathogen's radial development in dual inoculation

Antagonism between the fungal isolates was observed for 10 consecutive days and ranked on a scale of 1-5 as previously described by Mallikarjuna and Gowdu.[27]

Where:
1 = The antagonist completely engulfed the isolate and the whole surface of the medium.
2 = The antagonist outgrew the medium surface by at least two-thirds.
3 = Neither the antagonist nor the isolate appeared to dominate the other, with each colonizing half of the medium-surface.
4 = The isolate appeared to be in control of the antagonist and had at least colonized two-thirds of the medium-surface.
5 = The isolate utterly outgrew the antagonist, taking up the entire medium-surface.

Statistical Analysis

Analysis of Variance (ANOVA) and tests of least significant difference (LSD) at $P \leq 0.05$ were used to separate means

Results and Discussion

Physicochemical attributes of the experimental soil

The soil pH, moisture content, amount of organic matter and the water holding capacity of the soil before the planting of soya bean varieties were 6.00, 11.86%, 0.41% and 32.49 mg/g, respectively. The soil texture was loamy soil. All the attributes tested except soil texture had slight change at soya bean maturity, pH (6.10), moisture content (9.53%), amount of organic matter content (1.16%) and the water holding capacity (23.21ml/g) as shown in Table 1. The physical-chemical properties of the experimental soil before sowing were optimal for the growth of soya bean seeds. The analysis of the physical-chemical properties of the experimental soil showed that the pH of the soil was moderate to slightly acidic. Studies have revealed that most plants thrive best on soil that is mildly to slightly acidic, because in this soil condition, majority of the substances containing plant nutrients have the highest solubilities.[16,28] Compared to other legume crops, soya beans are also more tolerant to soil types with a lower pH, however a pH below 5.2 interferes with nitrogen fixation.[29] The moisture content and the water holding capacity of the soil reduced at soya bean maturity. Sule and Oyeyiola,[4] observed this type of reduction in their studies with the cassava cultivar TMS 30572. According to Shivanna and Vasanthakumari,[19] microbial oxidation of the amount of soil organic matter occurs more frequently in soils with low moisture content than in soils that are constantly wet or dry. The water-holding capacity of soil depends on its organic matter content, soil texture and soil structure.[4 30-31] It was also observed that the moisture content in the soil depends on the amount of rainfall, the time interval between the rainfall

and soil collection, soil type, season of the year, soil temperature, vegetative cover, and organic content. The organic matter content in the rhizosphere soil increased. This could be due to the network of fungal hyphae in the soil. The soil texture of the study area was loamy sand which was found to be favourable for the growth of soya bean. Soybeans can be grown in a wide range of soil types, but the best soil is a deep, fertile loamy sand with excellent drainage.[29]

Identification and characterization by molecular methods of the fungal isolates

A total of eighteen fungal species were isolated from the rhizosphere soil of soya beans. They were identified as *A. niger, A. fumigatus, A. flavus, A. tamari, A. hortai, A. nidulans, A. arcoverdensis, R. delemar, P. pinophilum, P. lilacinus, B. proliferans, T. pinophilus, N. fischeri, B. cinerea, M. micheli, F. oxysporum, H. solani,* and *T. asperellum.* The macro and micro characterization of the isolated fungi are shown in Figure 1.

Table 1: Physical-chemical properties of the soil samples before planting and at Soya bean maturity

Physicochemical properties	Soil samples before planting	Soil samples at soya bean maturity
pH	6.00	6.10
Moisture content (%)	11.86	9.53
Organic matter content (%)	0.41	1.16
Water Holding Capacity(ml/g)	32.49	23.21
Soil Texture	Loamy soil	Loamy soil

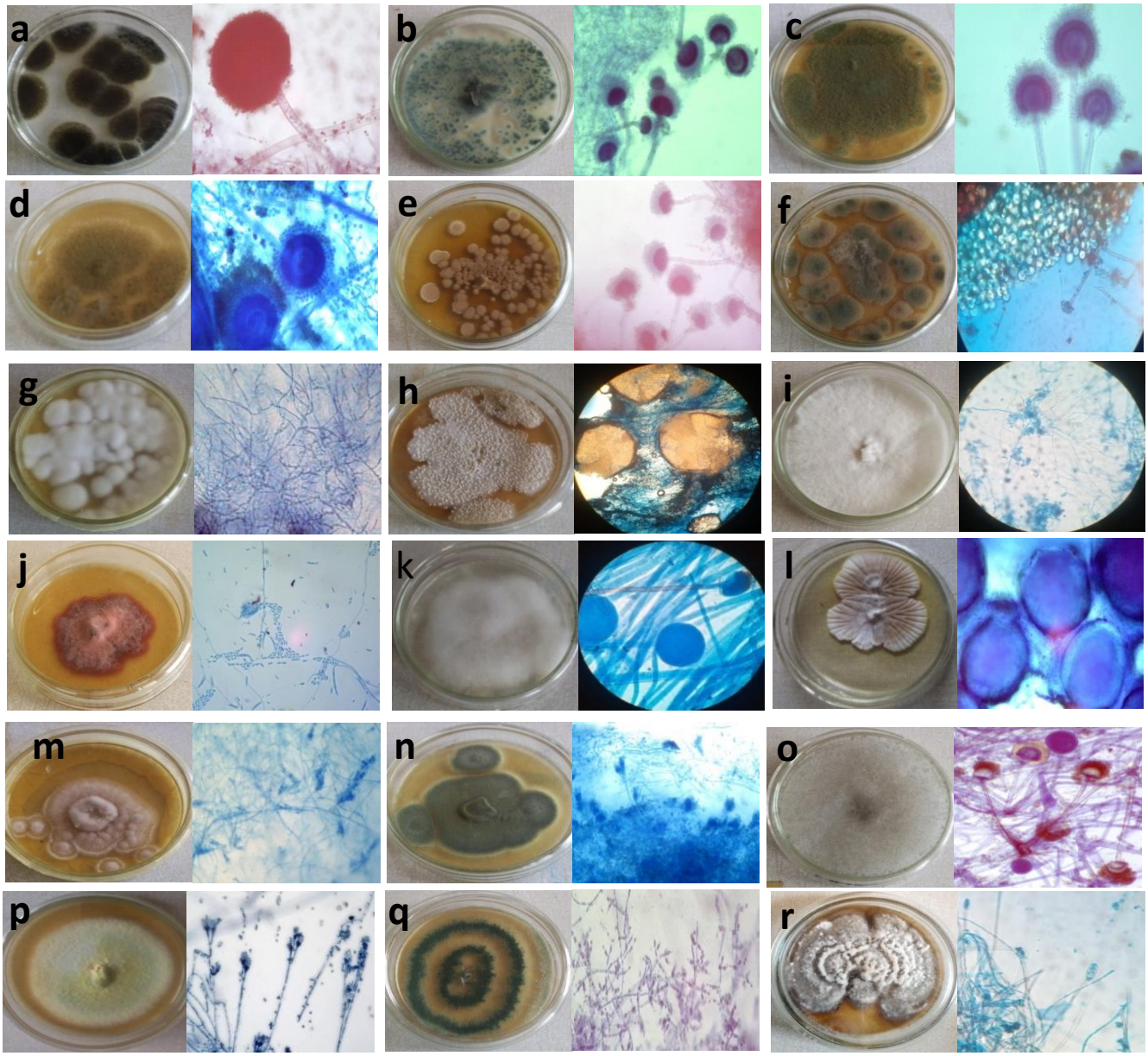

Figure 1: Morphology and photomicrograph of the fungal isolates (a-r x40). a. *Aspergillus niger* b. *Aspergillus fumigatus* c. *Aspergillus flavus* d. *Aspergillus tamari* e. *Aspergillus hortai* f. *Aspergillus nidulans* g. *Aspergillus arcoverdensis* h. *Blastobotrytis proliferans* i. *Botrytis cinerea* j. *Fusarium oxysporum* k. *Mucor micheli* l. *Neosartorya fischeri* m. *Paecilomyces lilacinus* n. *Penicillium pinophilum* o. *Rhizopus delemar* p. *Talaromyces pinophilus* q. *Trichoderma asperellum* r. *Helminthosporium solani*

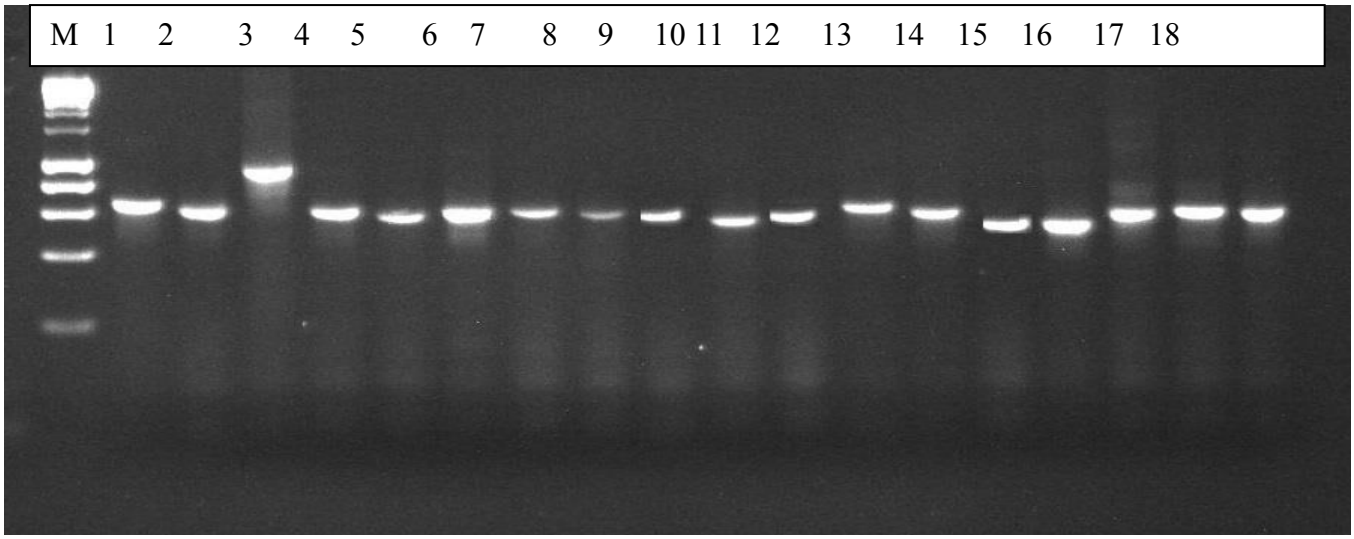

Figure 2: PCR amplification of 18S rDNA gene for the fungal isolates. Lane M: marker (100 bp) ladder; Lane 1-18: fungal isolates

Table 2: Isolates with the percentage sequence homology and their accession numbers

Isolates	% Sequence homology	NCBI Accession Number
Aspergillus niger	100.00	EU440768
Aspergillus fumigatus	99.30	HQ026746
Aspergillus flavus	94.70	KY911156
Aspergillus tamarii	100.00	KX010797
Aspergillus hortai	99.50	KP987087
Aspergillus nidulans	99.20	AB248971
Aspergillus arcoverdensis	99.40	KY808748
Blastobotrys proliferans	99.80	KM056341
Botrytis cinerea	99.70	LT629240
Fusarium oxysporum	99.80	FR731133
Mucor micheli	99.80	MG195972
Neosartorya fischeri	92.90	LC010248
Paecilomyces lilacinus	99.40	AB103380
Penicillium pinophilum	87.60	GU566245
Rhizopus delemar	99.50	KF710007
Talaromyces pinophilus	97.60	KJ207414
Trichoderma asperellum	100.00	JN108917
Helminthosporium solani	99.50	FJ487932

At maturity, the rhizosphere of soya beans yielded 12 fungal genera and 18 species in total. This finding is in line with the result of other studies that isolated numerous and diverse microflora in the rhizosphere of various plants.[32,33,19,34] For example, Eze and Amadi,[18] isolated 15 fungi in the rhizosphere of tomato seedlings. Some of their isolates such as *Rhizopus*, *Aspergillus*, *Penicillium*, *Trichoderma*, *Botrytis* and *Fusarium sp* were also isolated in the present study. While investigating the fungi living in the soil of the rhizosphere, rhizoplane, and non-rhizosphere of the root region of the cassava cultivar TMS 30572, Sule and Oyeyiola,[4]discovered 25 species of fungi. Many of these species were isolated during research with soya bean. A total number of 12 species including *A. flavus*, *A. niger*, *Mucor*, *Paecilomyces*, *Rhizopus* and *Trichoderma species* were isolated by Dawar *et al*,[3] from the rhizosphere of *Amaranthus viridis* L. while Olanrewaju *et al*,[35] reported discovering 12 fungi from okra plant's rhizoplane. Devi and Chhetry,[26] reported greater spectrum of fungal species in the rhizosphere of pigeon pea. They attributed their findings to the availability of nutrients for fungal growth released by root exudates around the rhizosphere. *Aspergillus niger* and *Penicillium chrysogenum* were isolated by David *et al*,[36] from the rice plant (Oryza sativa Linn) rhizosphere and identified via culture and molecular techniques. The isolates were further characterized by the PCR amplification of 18S rDNA gene using the ITS universal primer set, which included ITS 1: 5'TCC GTA GGT GAA CCT GCG G 3' and ITS 2: TCC TCC GCT TAT TGA TAT GC 3'. The amplification product of 100bp obtained for all the isolates are as shown in Fig. 2. The isolates with the percentage sequence relatedness of the already identified fungi in the GeneBank are shown in Table 2. To the best of our knowledge, this is the first study on the molecular characterization of the fungal isolates from the rhizosphere of soya beans grown in Enugu State, Nigeria. Results indicate that numerous mycoflora abound in the rhizosphere of the soya bean crop.

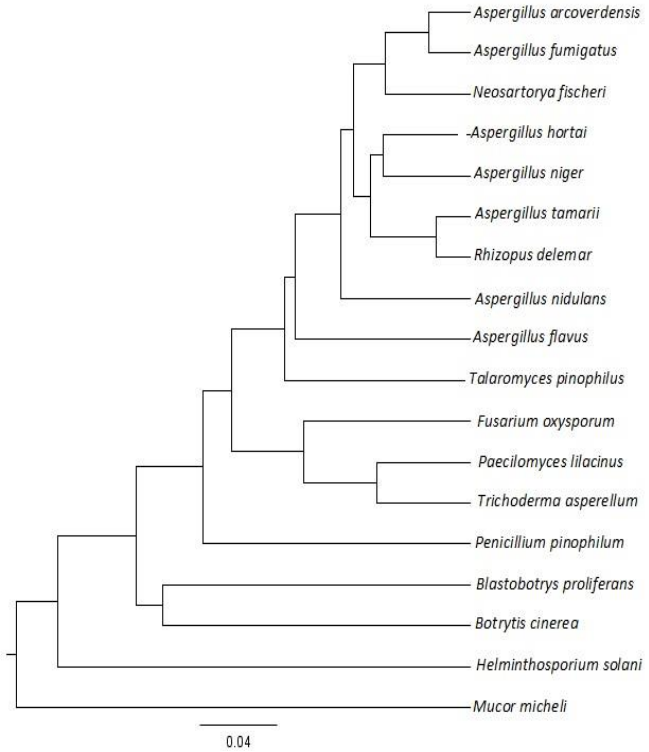

Figure 3: Phylogenetic tree of the fungi isolated from the rhizospheric soils of soya beans.

Table 3: Percentage occurrence of the fungal rhizosphere population

Fungal Isolates	Number of occurrences	Frequency of occurrence (%)
A. niger	25	15.63
A. fumigatus	02	1.25
A. flavus	08	5.00
A. tamarii	10	6.25
A. hortai	07	4.38
A. nidulans	07	4.38
A. arcoverdensis	06	3.75
R. delemar	12	7.5
H. solani	06	3.75
P. pinophilum	02	1.25
P. lilacinus	03	1.88
B. proliferans	05	3.13
T. pinophilus	14	8.75
M. micheli	18	11.25
N. fischeri	06	3.75
B. cinerea	11	6.88
F. oxysporum	15	9.38
T. asperellum	03	1.88

The phylogenetic tree of the fungal isolates shows *M. micheli* at the root of the evolutionary lineage from which other isolates tend to have evolved (Figure 3) Some species were found to be more closely related than others. For example, *A. arcoverdensis* was found to be more closely related to *A. fumigatus* whereas *A. hortai* tended to be closely related to *A. niger*. The phylogenetic tree of the rhizosphere fungi isolated in this study indicated that comparable terrestrial isolates are known to exist, and the species underwent a minor amount of evolution to help ensure they survive. The degree of similarity to earlier fungi indicated that the fungi species had not been opened to elements that would have encouraged greater genetic diversification.[37] The percentage sequence relatedness of the isolates with species in the GeneBank is also presented in Table 3.

Fungal rhizosphere populations

The percentage of occurrence of all the fungal isolates from the rhizosphere soil of soya bean is presented in Table 3. *Aspergillus niger* were found to be predominant in the rhizosphere soil of soya bean with a frequency of occurrence of 15.63% followed by *M. micheli* with the frequency of occurrence of 11.25% and the least abundant was *A. fumigatus* with 1.25% frequency of occurrence. In this study, *A. niger* was the most abundant species found in the rhizosphere of soya beans. Similar to the study, Abdel-Hafez *et al*.[38] stated that the wheat plant most common rhizosphere fungi were *A. niger*, *Aspergillus* species and *Fusarium* species. Also Abdel-Nasser *et al*.[39] showed the prevalency of *Aspergillus* species in the rhizosphere of different cultivated plants. The dominance of *Aspergillus* species could be as a result of their heavy sporulating habit and their capacity to produce antibiotics.

Antagonism between Trichoderma asperellum and the other fungal isolates

The percentage inhibition and dual culture interaction of the antagonistic fungus and the other fungal isolates are shown in Figures 4 and 5. The antagonist *T. asperellum* showed different percentages of inhibition in all the isolates tested. However, the inhibitory effect of *T. asperellum* on *Botrytis cinerea* was significantly higher at $p<0.05$ (80 %) than all the other isolates tested. The antagonist displayed lower inhibition percentages on the *A. niger* (49 %), *A. flavus* (38 %) and *F. oxysporum* (35 %). The least inhibited isolate was *R. delemar* with less than 10 % inhibition (Figure 5). The degree of interaction based on the ranking of *Trichoderma asperellum* with rhizosphere fungal isolates of Soya bean is presented in Table 4. *Botrytis cinerea*, *Blastobotrys proliferans* and *Paecilomyces lilacinus* were completely colonized by the antagonist on the 6th, 7th and 9th day respectively. *Rhizopus delemar* and *Penicillium pinophilum* colonized two-thirds of the medium on the 4th and 8th day, respectively. Antagonist overgrew two-thirds of the medium surface for all the other isolates and more were on the 6th day. The dual culture interaction of the antagonistic fungus on the other fungal isolates showed that *Trichoderma asperellum* suppressed almost all the other isolates with a maximum number of the pairwise comparisons indicating the fungus to have the higher competitiveness than the other isolates. This may be attributed to several alkaloids and terpenes produced by *T. asperellum*.[40] Competition, antibiosis, and mycoparasitism could all play a role in *Trichoderma* sp possible antagonistic behavior, according to Ozbay and Newman,[41]. Additionally, *Trichoderma* species produce antibiotics like gliotoxin and viridin as well as cell wall disintegrating enzymes and physiologically active heat-stable metabolites like ethyl acetate, which are known to be implicated in the suppression of disease occurrence.[26,42] *T. asperellum* showed more antagonistics activity on the fungal isolates on the sixth day post inoculation than on the other days. Overgrowth interaction was observed with *Botrytis cinerea* (6th day), *A. arcovendensis* (6th day), *Blastobotrytis proliferans* (7th day) and *A. tamarii* (9th day). Mallikarjuna and Gowdu[27] in their study on *in vitro* screening of rhizosphere fungi for bio control of *Macrophomina phaseolina* observed most of the overgrowth antagonistic interaction on the 7th day and complete or significant suppression of the isolates by the antagonist on the 14th day. The inhibitory interaction of *Mucor micheli* was noted on the 6th day and rated 2 as the antagonist colonized two-third of the culture plate in a contact interaction. Devi and Chhetry,[26] also observed a contact interaction between *Trichoderma* sp and *Fusarium udum* on the 3rd day. In the present study, inhibition at a distance was observed in *A. nidulans, A. hortai, A. niger, F. oxysporum, Paecilomyces lilacinus,* and *Talaromyces pinophilus* as a clear zone of inhibition was observed without physical contact between the colonies. This implies that the antagonist secretes a diffusible, non-volatile inhibitory chemical. Similar to this study, Mallikarjuna and Gowdu,[27] observed a clear zone of inhibition between *Macrophomina phaseolina* and *A. flavus*. AL-Saeedi and AL-Ani,[7] also reported a clear zone of inhibition between *Trichoderma harzianum* and *Aspergillus* sp.

Conclusion

The results of this study imply that the soya bean rhizosphere soil contains a diversified fungal community which could be due to plant root exudates. Therefore, it is crucial to carry out further study on the role of plant root exudates in growth stimulation of fungi with the aim of identifying the specific exudates. The results from such studies could be exploited in promoting the growth of beneficial organisms. This study has also provided a baseline of fungal communities that could be found in the rhizosphere soils of soya beans. The molecular characterization authenticated the phenotypic identities of the fungal isolates. Designing efficient and secure biocontrol techniques requires knowing the mechanisms underlying *Trichoderma asperellum* antagonistic action on phytopathogens as well as the choice of biological control agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

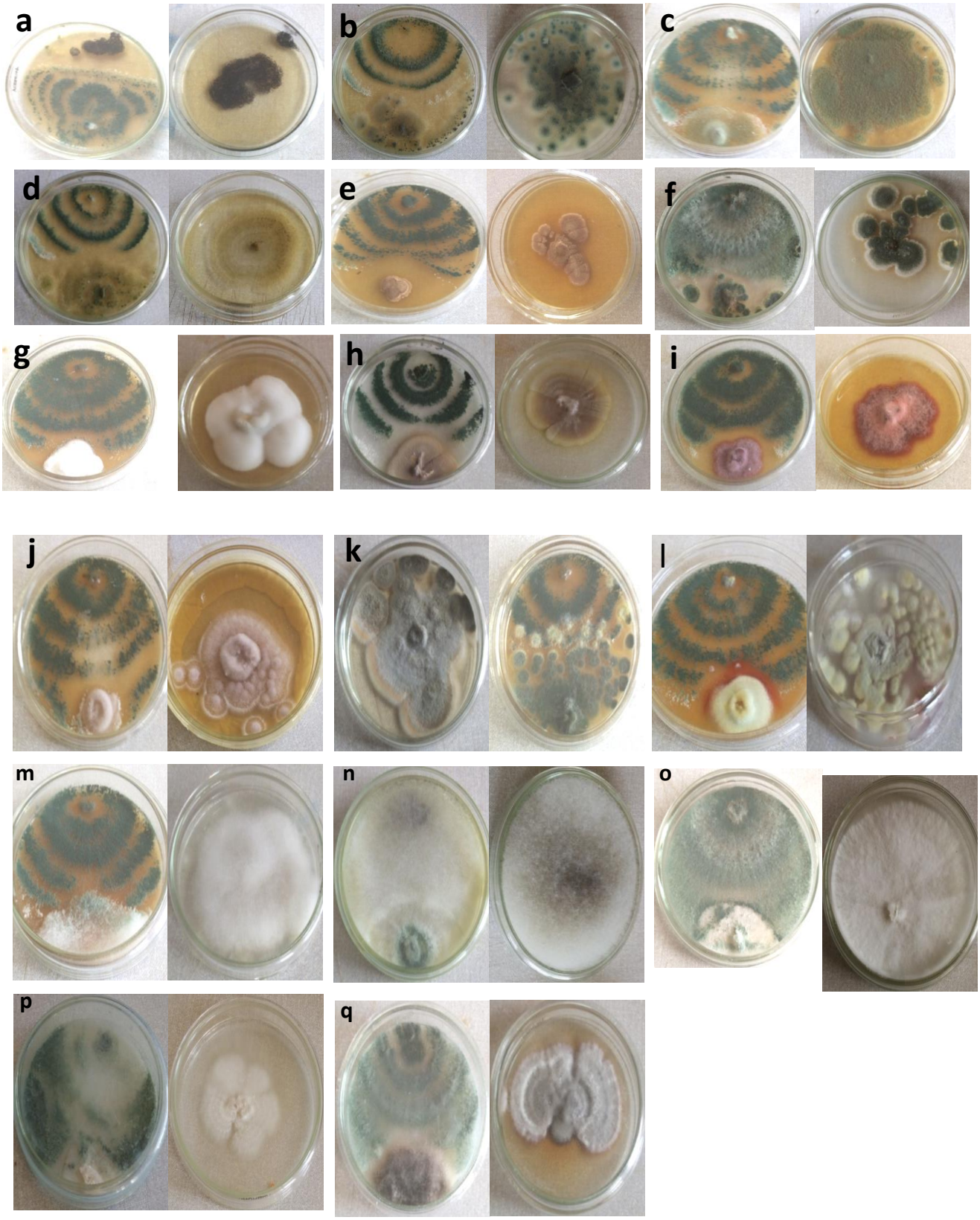

Figure 4: Inhibition of fungal growth due antagonism (a-q x40). (a: *T. asperellum+A. niger*, b: *T. asperellum+A. fumigatus*, c: *T. asperellum+A. flavus*, d: *T. asperellum+A. tamarii*, e: *T. asperellum+A. hortai*, f: *T. asperellum+A. nidulans*, g: *T. asperellum+A. arcoverdensis*, h: *T. asperellum+N. fischeri*, i: *T. asperellum+F. oxysporum*, j: *T. asperellum+P. lilacinus*, k: *T. asperellum+P. pinophilum*, l: *T. asperellum+T. pinophilus*, m: *T. asperellum+M. micheli*, n: *T. asperellum+R. delemar*, o: *T. asperellum+B. cinereal*, p: *T. asperellum+B. proliferans*, q: *T. asperellum+H. solani*).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

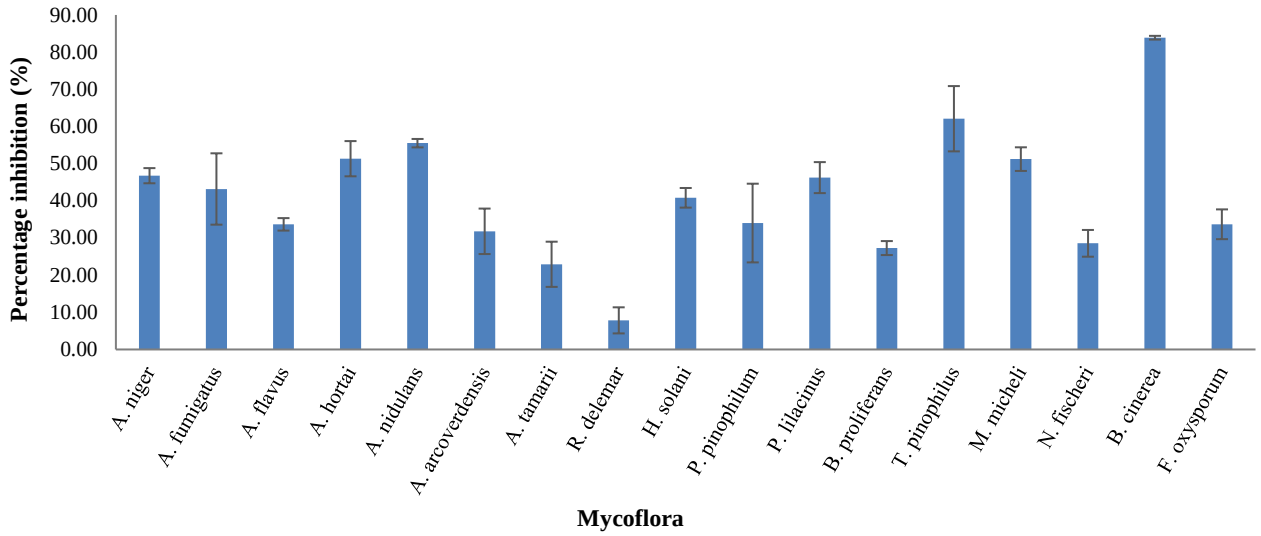

Figure 5: Percent growth inhibition of the rhizosphere fungal isolates 10 days post inoculation with *T. asperellum*

Table 4: Degree of interaction based on ranking of *Trichoderma asperellum* with rhizosphere fungal isolates of Soya bean

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Interaction of *Trichoderma asperellum* with the isolates			Ranking Scale							
A. niger						2				
A. fumigatus						2				
A. flavus						2				
A. tamarii									2	
A. hortai										2
A. nidulans						2				
A. arcoverdensis						2				
B. proliferans							1			
B. cinerea							1			
F. oxysporum				2						
M. micheli						2				
N. fischeri						2				
P. lilacinus									1	
P. pinophilum								4		
R. delemar				4						
T. pinophilus						2				
H. solani						2				

1 = The antagonist completely engulfed the isolate and the whole surface of the medium.
2 = The antagonist outgrew the medium surface by at least two-thirds.
3 = Neither the antagonist nor the isolate appeared to dominate the other, with each colonizing half of the medium-surface.
4 = The isolate appeared to be in control of the antagonist and had colonized at least two-thirds of the medium-surface.
5 = The isolate utterly outgrew the antagonist, taking up the entire medium-surface.

References

1. Kirk JL, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H, Trevors JT. Methods of studying soil microbial diversity. J Microbiol Methods. 2004; 58:169-188.
2. Prescott LM, Harley JP, Klein DA. The epidemiology of infectious disease. In: Microbiology. Prescott LM, Harley JP, Klein DA(Eds). McGraw-Hill, New York, USA; 2005. 821-843 p.
3. Dawar S, Batool M, Tariq M, Zaki MJ. Mycoflora in the rhizosphere of some wild plants around Karachi University Campus. Pak J Bot. 2014; 46(1):369-373.
4. Sule IO, Oyeyiola GP. Fungi in the rhizosphere and rhizoplane of cassava cultivar TME 419. Inter J. Appl Bio Res. 2012; 4:18 – 30.

5. Philippot L, Raaijmakers JM, Lemanceau P, Vander WH. Going back to the roots: The microbial ecology of the rhizosphere. Nat. Rev Microbiol. 2013; 11: 789-799.
6. Sylvia D, Fuhrmann J, Hartel P, Zuberer D. Principles and Applications of Soil Microbiology, Pearson Education Inc. New Jersey; 2005.
7. Al-Saeedi SS, Al-Ani BM. Study of Antagonistic Capability of *Trichoderma harzianum* Isolates against some Pathogenic Soil Borne Fungi. Agric Biol J. North Amer. 2014; 1:15-23.
8. Zin NA, Badaluddin NA. Biological function. of *Trichoderma* spp for agricultural applications. Annl Agric Sci. 2020; 65:168-178.
9. Raaijmakers JM, Leeman M, Van Oorschot MP, Van der Sluis I, Schippers B, Bakker PA. Dose-response relationships in biological control of *Fusarium* wilt of radish by *Pseudomonas* spp. Phytopathol. 1995; 85:1075-1081.
10. Haram S, Schickler H, Oppenheim A, Chet I. Differential expression of Trichoderma harzianum chitinases during mycoparasitism. Phytopathol. 1996; 86:980- 985.
11. Raaijmakers JM, de Bruijn I, de Kock MJ. Cyclic lipopeptide production by plant-associated *Pseudomonas* species: diversity, activity, biosynthesis and regulation. Mol Plt-Microbe Inter. 2006; 321: 341-361.
12. Harman GE, Petzoldt R, Comis A, Chen J. Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola.* Phytopathol. 2004; 94:147-153.
13. Verma M, Brar SK, Tyagi, RD, Surampalli, RY, Valero, JR. Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. *Biochem Engn J*. 2007; 37:1-20
14. Uguru MI, Oyiga BC, Jandong EA. Response of some soybean genotypes to different soil pH regimes in two planting seasons. Afr J. Plt Sci Biotech. 2012; 6:26-37.
15. Dugje IY, Omoigui LO, Ekeleme F., Bandyopadhyay R, Lava KP, Kamara AY. Farmers' Guide to Soybean Production in Northern Nigeria. IITA, Ibadan, Nigeria; 2009. 21 p
16. Oyeyiola GP. Rhizosphere mycoflora of okra (*Hibiscus esculentus*). Res J. Soil Biol. 2009; 1: 31-36.
17. Vibhute AD, Kale KV, Mehrotra SC, Dhumal RK, Nagne AD. Determination of soil physicochemical attributes in farming sites through visible, near-infrared diffuse reflectance spectroscopy and PLSR modeling. Ecol Proc. 2018; 7:26
18. Eze CS, Amadi JE. Studies on rhizosphere and rhizoplane microflora of Tomato (*Lycopersicon esculentum* Mill) seedlings. Inter J. Engn Sci Res. 2014; 3 (7): 666-672.
19. Shivanna MB, Vasanthakumari MM. Temporal and spatial variability of rhizosphere and rhizoplane fungal communities in grasses of the subfamily Chloridoideae in the Lakkavalli region of the Western Ghats in India. Mycosph. 2011; 2: 255-271.
20. Ogunleye AO, Ayansola OT. Studies of some isolated rot-causing mycoflora of yams (*Dioscorea* spp). Amer J Microbiol Biotechnol. 2014; 1: 9-20.
21. Barnett HL, Hunter BB. Illustrated Genera of Imperfect Fungi. The American Phytopathological Society, St. Paul, Minnosta, USA; 1999. 218 p.
22. Alexopoulos CJ, Mins CN, Blackwell M. Introductory Mycology. John Wiley and Sons Singapore; 2002. 869 p.
23. Agrios GN. Plant Pathology. Academic Press, New York; 2005. 922 p.
24. Ellis D, Davis S, Alexious H, Handke R, Bartley R. Descriptions of Medical Fungi. Mycology Unit Women Hospital North Adelaide, Australia; 2007. 198 p.
25. Srideepthi R, Lakshmisahitya U, Peddakasim D, Suneetha P, Krishna M. Morphological, Pathological and Molecular Diversity of *Colletotrichum capsici* inciting fruit rot in chilli (*Capsicum annuum* L.). Res J Biotech. 2017; 12:14-21.
26. Devi TR, Chhetry GK. Rhizosphere and non-rhizosphere microbial population dynamics and their effect on wilt causing pathogen of pigeon pea. Inter J. Sci Res Pub. 2012; 2:2250-3153.
27. Mallikarjuna M, Gowdu BJ. Isolation, identification and *in vitro* screening of rhizospheric fungi for biological control of *Macrophomina phaseolina*. Asian J Plt Pathol. 2015; 9: 175-188.
28. Kellogg CE. Soil. In: Bayer P, Feinberg B, Tantillo MD, Towers VF, Cole BL, Peckaitis CM, Tench HG (eds). The Encydopedia Americana, *Vol. 25*, Grolier Incorporated, Danbury, Connecticut; 1998. 176-186 p.
29. International Institute of Tropical Agriculture (IITA). Farmers' guide to soybean production in Northern Nigeria. IITA, Ibadan, Nigeria; 2009. 21 p.
30. Baker S. *Aspergillus niger* genomics: Past, Present and into the future. Med Mycol. 2006; 44: 17-21.
31. Olahan GS, Sule IO, Garuba T, Salawu YA. Rhizosphere and Non-Rhizosphere Soil mycoflora of *Corchorus olitorius* (Jute). Sci World J. 2016; 11: 23-26.
32. Cavaglieri L, Orlando J, Etcheverry M. Rhizosphere microbial community structure at different maize plant growth stages and root locations. Microbiol Res. 2007; 164 (4): 391 – 399.
33. Bhat PR, Kaveriappa KM. Rhizoplane mycoflora of some species of Myristicaceae of the Western Ghats, India. Inter Society Trop Ecol. 2012; 52: 163-175.
34. Bharti M, Pravesh R. Studies on the rhizosphere and non-rhizosphere mycoflora of *Lygodium flexuosum* (L.) Sw. and *Ampelopteris prolifera* (Retz.) copel of Ranchi district of Jharkhand. Inter Quarterly J. Environ Sci. 2012; 1: 61-68.
35. Olanrewaju SO, Akinro EB, Oladipupo OA. Fungal colonization of the Rhizoplane of okra (*Hibiscus esculentus*) plant. J. Environ Sci, Toxicol Food Technol. 2014; 8: 2319-2402.
36. David OM, Olawusi AC, Oluwole OA, Adeola PO, Odeyemi AT. Isolation, Molecular Characterization and Application of *Aspergillus niger* and *Penicillium chrysogenum* with Biofertilizer Potentials to Enhance Rice Growth. Trop. J. Natr. Prod. Res. 2023; 7(4): 2790-2795.
37. Ganley AR, Kobayashi T. Highly efficient concerted evolution in the ribosomal DNA repeats. Total rDNA repeat variation revealed by whole-genome sequence data. Gene Res. 2007; 17:184-191.
38. Abdel-Hafez SI, Ismail MA, Hussein NA, Abdel-Hameed NA. Fusaria and other fungi taxa associated with rhizosphere and rhizoplane of Lentil and Sesame at different growth stages. Acta Mycologic. 2012; 47: 35-48.
39. Abdel-Nasser AZ, Elkhateeb WA, Mazen MB, Hashem M, Daba GM. Survey of all mycobiota associated with rhizosphere and rhizoplane of different cultivated plants in new reclaimed soil, upper Egypt, and examination of the most common fungal isolates to produce mycotoxins. Egyptian Pharm J. 2016; 13: 64-70.
40. Sharma R, Rajak RC, Pandey AK. Evidence of antagonistic interactions between rhizosphere and mycorrhizal fungi associated with *Dendrocalamus strictus* (Bamboo). J Yeast and Fungal Res. 2010; 1:112-117.
41. Ozbay N, Newman SE. Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. Park J Biol Sci. 2004; 7:478-484.
42. Mujeebur KR, Shahana MK, Mohiddin FA. Biological control of *Fusarium* wilt of chickpea through seed treatment with the commercial formulation of *Trichoderma harzianum* and or *Pseudomonas fluorescens*. Phytopathol Mediterr. 2004; 43: 20-25.