AQUATIC PHYCOMYCETES AND ASCOMYCETOUS FUNGI ISOLATED FROM *ARTEMISIA ANNUA* L. PLANTATION SOIL IN A NIGERIAN UNIVERSITY

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Abstract

Survey was carried out on the ascomycetous and aquatic fungi present in the soil of University of Jos *Artemisia annua* Plantation in Gangnum, Langtang South Local Government Area of Plateau State. Portions of the soil samples collected from three (3) locations were steamed in glass beaker placed in a steamer for 4-10 minutes at 100^{o}C before inoculation. Fungal isolation was carried out using soil plate method incubated at 25 ± 2^{o}C. Aquatic phycomycetes were isolated using baits. Two (2) genera of aquatic phycomycetes were isolated, including *Achyla dubia* and *Allomyces arbuscular*. Fifty–eight ascomycetous fungi were also isolated from the soil samples. The predominant ascomycetous fungi isolated include among others; *Chaetomium bastrychodes, C. cancriodeum, C. cochloides, C. globosum, C. nigricolor, C. senegalensis, C. spirale, Aspergillus candidus A. flavus, A. fumigatus , A. glaucus, A. nidulans, A. niger, A. oryzae, A. terreus, F. avenaceum, F. oxysporum, F. roseum, F. solani, F. sporitrichioides, Penicillium chrysogenum, P. citrinum, P.notatum, P.expansum, Trichoderma harzianum, T. piluliferum, Alternaria alternata, Aureobasidium* pullulans, *Botrytis cinerea, Cladosporium* sp, *Curvularia lunata, Scopulariopsis* sp, *Torula herbarum,* Unidentified *sp and a* Basidiomycete. The physico-chemical properties of the soil samples were found to be varied, and were found to affect the distribution and population of fungi. The soil was found to be high in organic matter content which

could have been as a result of activities of the species of fungi numerous in the soil. The implications of the results are discussed.

Keywords: Fungi, Artemisia annua, Plantation, ascomycetous, Soil

Introduction

Soil is composed of five major components: mineral matter, water, air, organic matter and living organisms. The quantity of these constituents is not the same in all soils but varies with locality (Alexander, 1977). The soil being a complex environment is colonized by immense diversity of microorganisms. Soil microbiology focuses on the soil viruses, bacteria, fungi, actinomycetes and protozoa, but it has soil animals such as the nematodes, mites and others collectively referred to as the soil microbiota (Cruegar, 1989). The vast differences in the composition of soils, physical characteristics and the agricultural practices by which they are cultivated, result in corresponding large differences in the microbial population both in the numbers and kinds (Gokalp *et al*., 2010).

Fungi are eukaryotic organisms important to humans in both harmful and beneficial ways. They are among the most important microorganisms associated with food. They are distantly related to plants and more closely related to animals but rather different from either of those groups. About 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist (Hawksworth *et al.*, 1995). They are mainly multicellular organisms which composed of long and thread like cells connected end to end called hyphae which in mass constitute the mycelium. Some fungal species also grow as single cells. Their cell wall is rigid and largely composed of chitin. Most fungal species are non-motile and reproduce by means of spores. Both sexual and asexual spores may be produced depending on the fungal species and the environment (Kendrick, 1992).

Soil is a diverse medium composed of many minerals and substrates essential for metabolic pathways of prokaryotic and eukaryotic inhabitants (Handelsman, 2004). The abiotic and biotic diversity present in this medium makes it difficult for the isolation of all the microbial community present. That is why research has demonstrated that not even 1% of the entire soil microbial community has been identified (DeLong, 2002). There is great opportunity for discovering new groups of microorganisms with industrial and clinical importance in soil. After higher vegetation has become established, a continuum of soil processes produces the dynamic mixture of living and dead cells, soil organic matter (SOM), and mineral particles in sufficiently small sizes to permit the intimate colloidal interaction characteristic of soil (Andrew *et al*., 2008).

The *Artemisia annua* plantation soil of Gangnum in Langtang South Local Government of Plateau State has soil that is very rich in humus and so is good for planting and agricultural activities. Fungi has been known to play a significant role in the fertility of soil, their abundance and existence solely depend on the environmental (soil) conditions (Paul and Clark, 1996).

The present study intends to examine the physico-chemical characteristics of the plantation soil samples and to identify morphologically the mycoflora of the soil (plantation).

Materials and Methods

Study area

This study was carried out in the University of Jos *Artemisia annua* plantation situated at Gangnum in Langtang South Local Government of Plateau State (8^0 38'N, 9^0 48'E, 8.633^0 N, 9.8^0 E), Nigeria.

Collection of samples

Soil samples were collected from; University of Jos, *Artemisia annua* plantation soil at Gangnum Langtang South Local Govt. area of Plateau State at guided random with the help of sterile soil auger and hand trowel. The samples were collected from 3 locations in each site and then mixed together in order to obtain a representative sample.

A weight of 500g of soil was collected from depth of 10cm of each of the locations. The different soil samples were stored in sterile cellophane bags in the refrigerator (4^0C) and later plated out for the assessment of their fungal contents.

Ecological parameters of the soil samples.

pH, Moisture content (%), Organic content(%) texture and colour of the soil samples were also assessed.

Soil pH determination

The method of Ogbonna and Pugh (1983) was used for the assessment. A weight of 20 grams was weighed out from each soil sample and mixed with 100ml of sterile distilled water in 500ml conical flask. The content was thoroughly mixed to get a homogenous soil suspension. The suspension was kept for about one hour to settle. With the aid of Jenway pH meter model 3310, the average pH of each soil solution was then determined and recorded in triplicates.

Percentage moisture content determination.

The method of Ogbonna and Pugh (1983) was used for the assessment. A weight of 30 grams of soil from each soil sample was dried to

a constant weight in hot air oven set at 110^{o}C. The percentage moisture contents of the soil samples were determined in triplicates using equation:

$$\% \text{ moisture content} = \frac{\text{initial weight of soil (g)}}{\text{weight of dry soil taken}} \times \frac{100}{1} \quad (1)$$

Percentage organic content determination.

The method of Ogbonna and Pugh (1983) was used for the assessment. A weight of 30 grams of soil from each soil sample previously dried to a constant weight in hot air oven set at 110^{o}C was used in the determination of the percentage (%) organic content. The percentage organic content of the soil samples were determined in triplicates using the equation:

$$\% \text{ organic content} = \frac{\text{weight of soil organic content (g)}}{\text{weight of soil organic content}} \times \frac{100}{1} \quad (2)$$

Isolation of fungi from the different soil samples

The method employed was that described by Warcup in 1950 and adopted by Ogbonna and Pugh (1983). Portions of the soil samples were steamed in glass beaker placed in a steamer for 4-10 minutes at 100^{o}C as reported by Ogbonna and Pugh (1982). A weight of 0.03g of the steamed soil portions were then used to prepare soil crumb plates which were incubated at 25^{o}C. These were used for the isolation of ascomycetes. Other portion of the soil was placed in sterile water baited with hemp seeds for the isolation of aquatic phycomycetes.

A total of 40 sterile Petri dishes were used for the isolation work. Sterile molten Czapek Dox Agar and Sabouraud Dextrose Agar media were used for the isolation work Three drops of gentamycin (40mg/ml) were added into each culture plate in order to suppress the growth of bacteria. The plates were labeled appropriately.

The culture plates were divided into five batches of three plates each and were incubated at 25^{o}C for the isolation of mesophilic fungi as reported by Ogbonna and Pugh (1982). The plates were examined daily for fungal growth. The rough cultures were sub cultured severally in order to obtain pure cultures. The culture plates were examined under the microscope to determine the number of fungi colonies. Scorings were made for each fungus colony that appeared in the plate and the results were subjected to statistical analyses. The experiments were replicated five times.

Characterization of the fungal isolates

Each isolate was identified based on its colonial and cultural properties, and the microscopic features of its sporulating structures. The characteristics of the vegetative mycelia (hyphae) and color of the colonies

were observed over a period of 24 to 168 h. The morphology of the isolates, stained with lactophenol-cotton blue, was studied using a light microscope and compared according to the descriptions given by Thom and Raper (1945), and Samson *et al*., (1984).

Results and discussion

The ecological parameters of the soil samples including pH values, percentage moisture and organic matter contents, colour texture as shown in Table 1 revealed that the pH values of the sampled soils varied with location. It was found that location A was acidic with a pH value of 5.80 while location B recorded a high pH value of 6.62 indicating that the soil was slightly acidic. Location C had a pH value of 7.21 indicating that the soil was slightly alkaline. Location B had the highest moisture content of 1.6%, while the least value of moisture content obtained goes to Location C with 0.77%. In the case of organic matter content, Location A had the highest with 3.84% and the lowest value goes to Location C with 2.30%. However, differences in the soil pH, moisture content and organic matter content values of the different sampling locations were observed to be statistically significant. Similar results were obtained by Steffi and Josephine, (2013) in their work on Analysis of farm soil microbial profile, where they indicated that there was a correlation between the pH of soil in different locations. They reported that the soil pH of different types of soil samples in their study was near neutral ranges, which favours microbial growth.

Table 1: Ecological parameters of the soil samples

Soil sample	Soil pH	%moisture content	%Organic Content	Colour	Texture
A	5.80^{b}	0.72^{b}	3.84^{c}	Dark brown	Fine
B	6.62^{a}	1.60^{a}	3.67^{a}	Brown	Coarse
C	7.21^{c}	0.77^{b}	2.30^{a}	Light brown	Clay
SE±	**0.071**	**0.072**	**0.058**		

Means having the same superscript in the same column are not significantly different from each other at 5% probability level.

The pH of the soil samples were within the ranges that support fungi growth in culture. Fungi as a group tolerate a wide pH range, but some fungi are more tolerant to acidic soils. As compared to bacteria they can tolerate a wide range of pH 4-8 (Ogbonna and Pugh, 1982) which also correlates with the findings in this study (Table 1). As indicated in this study, location A which was acidic (pH 5.80), had the highest fungal plate count. This was followed by location B (pH 6.62). The slightly alkaline condition existing in location C could possibly be responsible for the low occurrence of most of the isolates.

The high organic content of the soil samples must have contributed to the diversity of fungi isolated from the locations. The organic matter contents of the soil which is the main source of utilisable substrates for the fungi plays a significant role as high number of the fungal species were recovered from locations A and B. Humus (organic matter) rich soils have large fungal population than soil poor in humus (Adams *et al*., 1999, Andrew *et al*., 2008).This also correlates with the findings in this study as shown in Table 1. The leaves and other agricultural wastes of *A. annua* are highly biodegradable and must have been easily decomposed by the array of fungi present on the Plantation soil. It is of interest to note that most of the ascomycetous fungi obtained in this study correlated with the physico-chemical characteristics of the locations from where they were isolated.

A total of 60 species of mesophilic fungi were isolated from the soil samples, which included two (2) aquatic Phycomycetes and 58 Ascomycetes. The aquatic Phycomycetes isolated included *Achyla dubia* and *Allomyces arbuscular*. The isolation of aquatic Phycomycetes could be as a result of seasonal ponds found intermittently at the Plantation.

The details of the fungal isolates are presented in Table 2 and the different fungal groupings are illustrated in Figure 1. Some of the fungal isolates are shown in figures 2a-2d.

Table 2: Species of Aquatic Phycomycetes and Ascomycetous fungi isolated from *A. annua* Plantation soils.

S/N	Fungal Isolates	SITES			Total	%FO
		A	B	C		
	Aquatic Phycomycetes					
1	*Achyla dubia* Coker	+	+	+	3	100
2	*Allomyces arbuscula* Butler	+	-	+	2	66.67
	Ascomycetes					
3	*Chaetomium bastrychodes* Zopf	+	-	+	2	66.67
4	*C. cancriodeum* Tschudy	+	+	-	2	66.67
5	*C. cochloides* Palliser	+	+	-	2	66.67
6	*C. globosum* Kunze	+	+	+	3	100
7	*C. nigricolor* Ames	+	+	-	2	66.67
8	*C. senegalensis* Ames	+	+	-	2	66.67
9	*C. spirale* Zopf	+	+	-	2	66.67
10	*Aspergillus candidus* Link	-	+	+	2	66.67
11	*A. flavus* Link	+	+	+	3	100
12	*A. fumigatus* Fres	+	+	+	3	100
13	*A. giganteus* Wehmer	-	+	-	1	33.33
14	*A. glaucus* Link	+	+	-	2	66.67
15	*A. longivesica* Huang and Raper	-	+	-	1	33.33
16	*A. nidulans* (Eidam) Wint	+	+	+	3	100
17	*A. niger* van Tieghem	+	+	+	3	100
18	*A. ochraceus* Wilhelm	+	+	-	2	66.67
19	*A. ornatus* Raper, Fernell and Tresner	+	-	-	1	33.33

20	*A. oryzae* Cohn	+	+	-	2	66.67
21	*A. ruber* Thom and Church	-	+	+	2	66.67
22	*A. scleretiorum* Huber	-	-	+	1	33.33
23	*A. stellatus* Curzi	+	+	-	2	66.67
24	*A. terreus* var. 1 Tirabosch	+	+	+	3	100
25	*A. terreus* var. 2 Kwon & Fernel	+	+	+	3	100
26	*Fusarium argillaceum* Saccardo	-	+	-	1	33.33
27	*F. avenaceum* Saccardo	+	+	-	2	66.67
28	*F. culmorum* Saccardo	+	-	-	1	33.33
29	*F. oxysporum* Schlechtendahl	+	+	-	2	66.67
30	*F. poae* Wollenweber	-	+	+	2	66.67
31	*F. roseum* Link	+	+	-	2	66.67
32	*F. solani* Appel & Wollenweber	+	+	-	2	66.67
33	*F. sporitrichioides* Sherbakoff	+	+	+	3	100
34	*Penicillium chrysogenum* Thom	+	+	+	3	100
35	*P. citrinum* Thom	+	+	+	3	100
36	*P.funiculosum* Thom	+	-	+	2	66.67
37	*P. fluorescens* Laxa	+	-	-	1	33.33
38	*P.notatum* Westling	+	+	-	2	66.67
39	*P.ochraceum* Bain	+	-	+	2	66.67
40	*P.patulum* Bain	+	+	-	2	66.67
41	*P.resticulosum* Birkinshaw, Raistrick and G. Smith	+	-	+	2	66.67
42	*P.rubens* Bourge (P. chrysogenum series)	+	+	-	2	66.67
43	*P.rubrum* Stroll, Bietr. Charakter (P. Pinphilum Hedgcock)	-	+	+	2	66.67
44	*P.expansum* Rapper &Thom	+	+	+	3	100
45	*Trichoderma hamatum* Bainier	-	-	+	1	33.33
46	*T. koningi* Oud	+	-	-	1	33.33
47	*T. piluliferum* Webster & Rafai	+	+	-	2	66.67
48	*T.harzianum* Rifai	+	+	+	3	100
49	*Alternaria alternata* keissler	+	+	-	2	66.67
50	*Aureobasidium* pullulans (de Bary) Arnaud	+	+	-	2	66.67
51	*Botrytis cinerea* Pers	+	+	+	3	100
52	*Cladosporium* sp	+	+	+	3	100
53	*Curvularia lunata* Beodij	+	+	-	2	66.67
54	*Graphium* sp.	-	-	+	1	33.33
55	*Helminthosporium* sp.	-	+	-	1	33.33
56	*Scopulariopsis brevicaulis* (Sacc.) Bainer	+	+	+	3	100
57	*Torula herbarum* Gray	+	+	+	3	100
58	*Ulocladium* sp.	-	-	+	1	33.33
59	Unidentified *sp.*	+	+	-	2	66.67
60	Basidiomycete	+	-	+	2	66.67
	TOTAL	48	46	31	125	

A, B, C = site locations from where the soil samples were collected %FO= Percentage frequency of occurrence

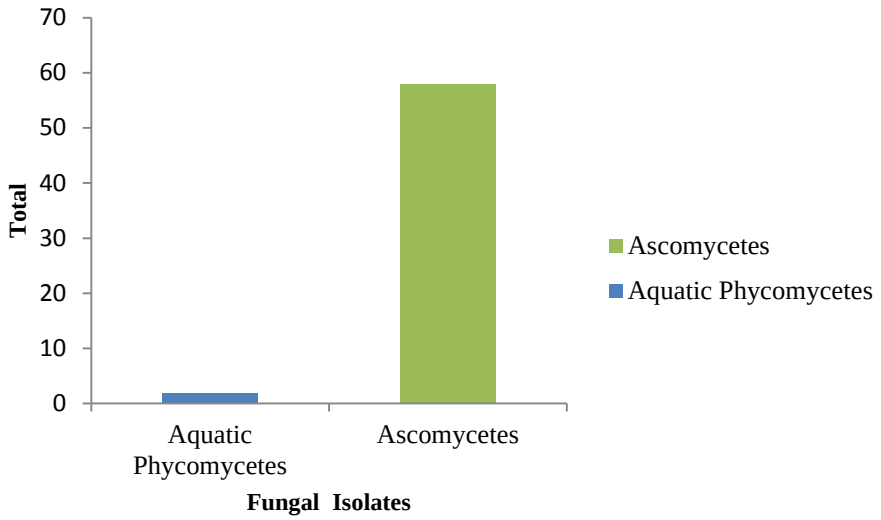

Figure 1: Aquatic Phycomycetes and Ascomycetous fungi isolated from *A. annua* Plantation soils

Table 3: Colonial and microscopic characteristics of some of the fungal isolates

Characteristics	*Aspergillus terreus*	*Scopulariopsis brevicaulis*	*T.harzianium*	*Curvularia lunata*
Morphology of reproductive structure	Conidiophore terminated in columnar vesicle. Biserriate phialides arose from the vesicle. Conidia borne on the phialides.	Branched conidiophore with conidial head that is brush-like. The conidia are spherical and very rough.	Irregularly branched conidiophore. phialospores in grape -like clusters.	Macroconidia are arranged in cluster at the tip of the conidiophores. They are curved and with 3 transverse septa.
Appearance on PDA medium / pigmentation	Sand brown Colour. Mycelia is white. Reverse is uncoloured.	Dull yellow colour to light brown with age. Reverse is light brown	Dull green colour. Reverse is uncoloured.	Dark brown and velvety. Reverse is near black.
Macroconidia	None	None	None	Abundant macroconidia which tapper at both ends
Hyphae	Septate	Septate	Septate	Septate

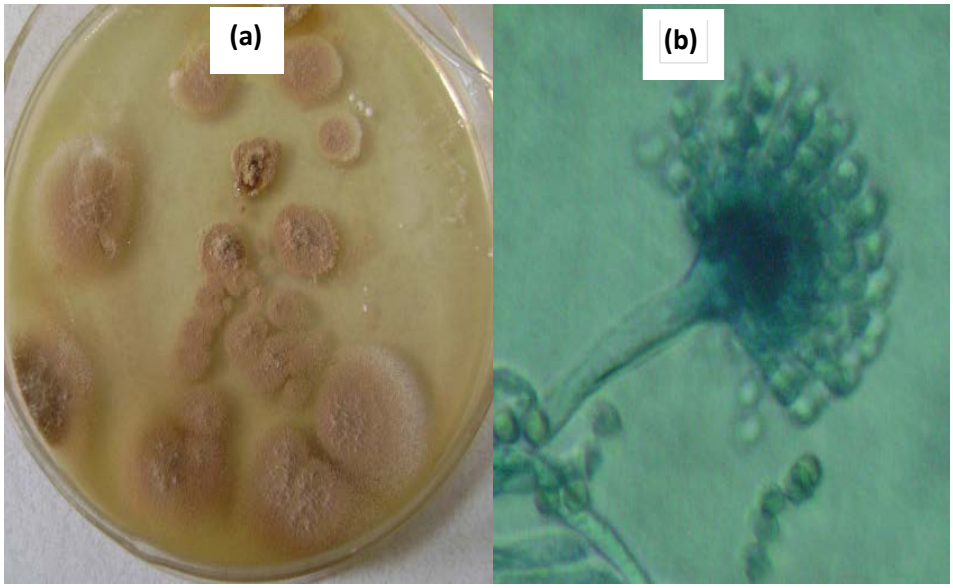

Figure 2a: *Aspergillus terreus* showing: (a) colony (b) conidial head with biserriate phialides (x1000)

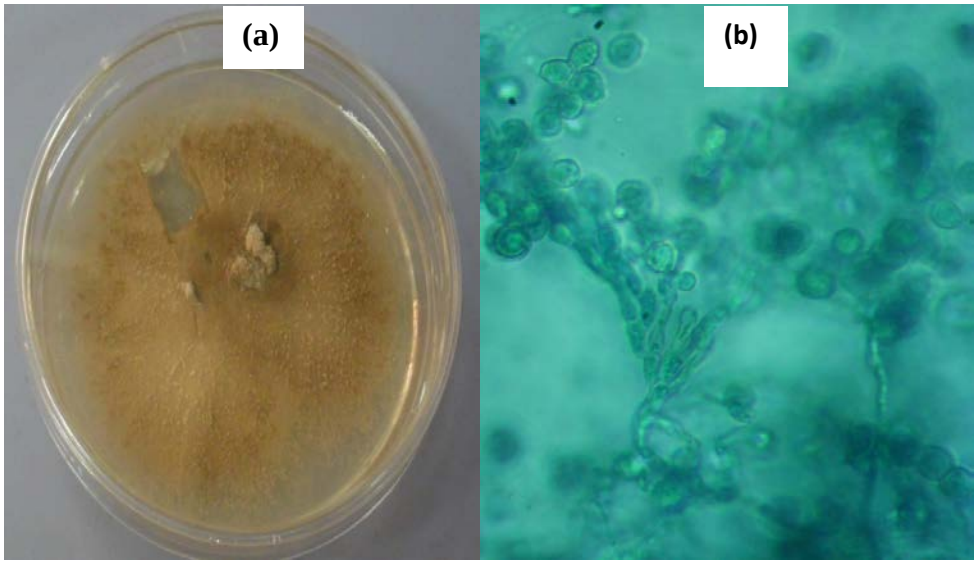

Figure 2b: *Scopulariopsis brevicaulis* showing: (a) colony (b) conidiogenous hypha with the rough conidia
in chain (x1000)

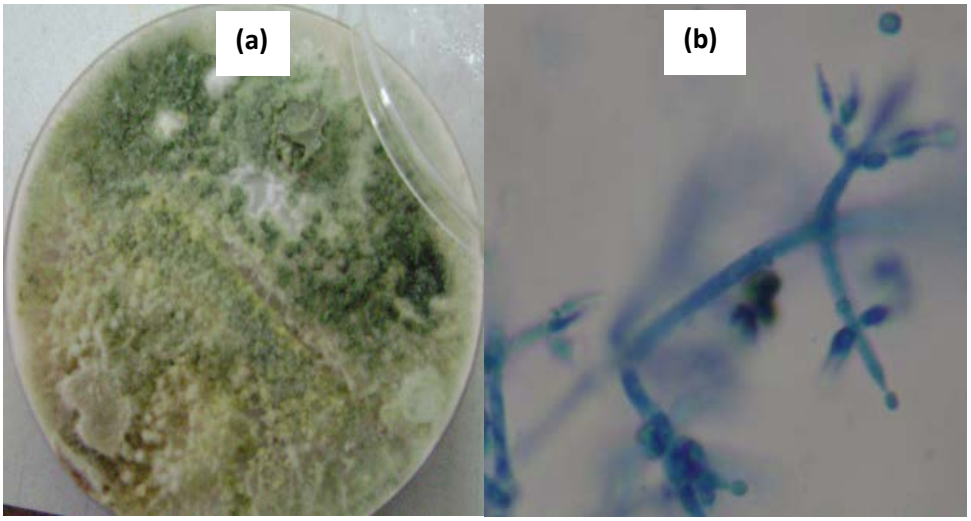

Figure 2c: *Trichoderma harzianum* showing (a) colony (b) Conidiophore, phialides and phialospore (x1000)

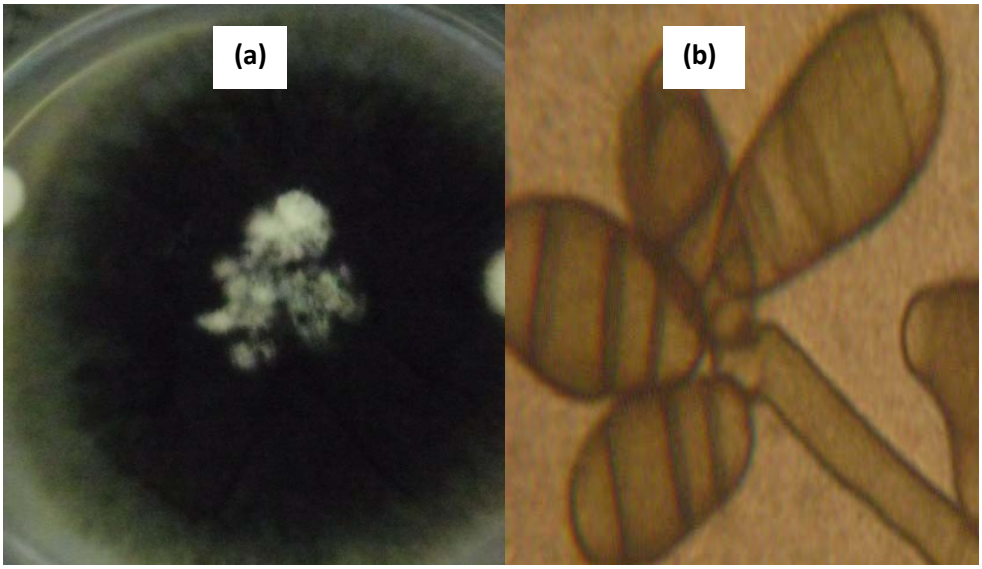

Figure 2d: *Curvularia lunata* showing (a) colony (b) conidiophores with macroconidia (x1000)

The findings of this research work indicated that there are lots of ascomycetous fungi present in the *A. annua* Plantation soils. Some of the groups of fungi encountered in this study have been obtained by previous workers, both in Nigeria and in other parts of the world. Nelson, (1988) reported the occurrence of *Trichoderma viride* and *Trichoderma album* (both of which are ascomycetes) in Douglas-fir-soil. The isolation of various fungal species showed that the agricultural soil was quite rich in microbial flora. This is similar to the reports of Amir and Pineau, (1998) and Okoh *et*

al., (1999). In Nigeria, soil fungi have been studied by Alabi (1967), Fajola *et al*. (1978) Ogbonna and Pugh (1982). *Aspergillus spp* (especially *A. niger) and Penicillium spp* were found to be frequently isolated and are of particular interest because of their public health importance. Patterson (1972) isolated four most common allergic fungi, *Alternaria, Aspergillus*, *Hormodendron*, *Cladosporium* and *Penicillium* based not only on incidence but on skin test reactivity. These groups of fungi except *Hormodendron* were also encountered in this study. Their common occurrence could be due to their high sporulating nature and also coupled with their ability to grow well on organic substrates, which were in abundance in the *A. annua* Plantation. *A. niger* is a common contaminant on various substrates. The employment of steaming of the soil at 100^{o}C before the isolation greatly expanded the list of the ascomycetous fungi. This finding is in consonance with the work done by Ogbonna and Pugh, (1982) and Ekundayo, (2004).

In conclusion, the University of *Artemisia annua* plantation soil in Gangnum, Langtang South Local Government of Plateau State has soil that is very rich in humus and so is good for both planting and agricultural activities.

References:

Adams, C. P., Bamford, K. M. and Early, M. P. (1990). *Principles of Horticulture* (3rd Ed.) Butterworth Heineman. p.25.

Alabi, R.O. (1967). Studies of some tropical aquatic phycomycetes found around Ibadan. M.Sc.dessertation, University of Ibadan.

Alexander, M. (1977). *Introduction to soil microbiology* 2nd Edition. Publ. John Wiley Sons Inc, p 3-4.

Amir H, Pineau R. (1998) Influence of plants and cropping on microbiological characteristics of some new Caledonian Ultramafic soils. Australian Journal of soil Resource 36 (3): 457 – 470.

Andrew. W.C, James. M. T. and Karen, H. (2008). Do fungi have a role as soil stabilizers and remediators after forest fire? *Forest Ecology and Management*. 257 (3)1063-1069.

DeLong, F. (2002). Microbial population genomics and ecology. *Current Opinion in Microbiology* 5: 520-524.

Ekundayo, E.O. (2004). Fungi in the rhizosphere and non-rhizosphere soil of Okra cultivated in southern Nigeria. *Nigerian Journal of Soil Science* 14: 48-55.

Fajola, A.O., Alasoadura, S.O. and Ogbonna, C.I. (1978). Some aquatic phycomycetes from riverine soils in Ibadan, Nigeria. *Nova Hedwegia* 29: 905-911

Gokalp, Z., M. Basaran, O. U. and Serin, Y. (2010). Spatial analysis of some physical soil properties in a saline and alkaline grassland soil of Kayseri Turkey. *African Journal of Agricultural Resources* 5: 1127-1137.

Gruegar, W. (1989). Biotechnology: *A textbook of Industrial Microbiology*. Publ. Sunderlan, Sinauer Association Inc. p. 430-433.

Handelsman, J. (2004). Metagenomics: application of genomics to uncultured microorganisms. *Applied and Enviornmental Microbiology* 70: 5522–5527.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C., and Pegler, D. N. (1995). Ainsworth and Bisby's *Dictionary of the Fungi* (9th Edition). CAB International, Wallingford, United Kingdom. 655pp.

Kendrick, B. (1992). *The Fifth Kingdom* (2nd Edition). Mycologue Publications, Ontario, Canada.386pp.

Nelson, E.E (1988). Occurrence of *Trichoderma* in a Douglas fir soil. *Mycopathologia* 74 (2): 280-284.

Ogbonna C.I.C. and Pugh, G.J.F. (1982). Nigerian Soil Fungi. *Nova Hedwegia* 36: 795-808.

Ogbonna, C.I.C. and Pugh, G.J.F. (1983). Thermophilic and Thermotolerant fungi from Nigeria. *International Biodeterioration Bulletin* 19 (2): 69-75.

Okoh L A, Badejo M A, Nathaniesl I T, and Tian G. (1999*)* Studies on the bacteria, fungi and springtails (collembola) of an agroforestry arboretum in Nigeria. *Pedobio* (43): 18: -27.

Patterson, R. A. (1972). Infestation of drytridiaceous fungi on phytoplankton in relation to certain environmental factors. *Ecology* 44: 416-429.

Paul, E. A and Clark, F. E (1996). *Soil Microbiology and Biochemistry*. 2nd Edition. (New York; academic press pp.225-229.

Samson, R.A., Hoekstra, E.S. and Van Oorschot, C.A.N.(1984). *Introduction to food-borne fungi*. Publ. Centraalbureau Voorschmmelculture baarn, Delft, Inst of Royal Netherlands Academy of Arts and sciences 249pp.

Steffi, S. and Josephine, R. M. (2013) Analysis of Farm Soil Microbial Profile International Journal of Research in Pharmaceutical and Biomedical Sciences 4 (1): 132-137.

Thom, C. and Raper, K. B. (1945). *A manual of Aspergilli*. Williams and Wilkins Co. Baltimore, USA. 373pp.

Warcup, J. H. (1950). The soil plate method for isolation of fungi from soil. *Nature 166*:117-118.