International Journal of Soil Mechanics & Foundations– IJSMF
Volume 2 : Issue 1 *[ISSN – 2475-272X]*
Publication Date: 30 October, 2015

DIVERSITY OF FUNGI FROM SOILS OF ARTEMISIA ANNUA L. PLANTATION IN A NIGERIAN UNIVERSITY

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Abstract-The Centre for Biotechnology and Genetic Engineering (CBGE), University of Jos *Artemisia annua* L. Plantation situated at Gangnum, Langtang South Local Government of Plateau State, Nigeria has been unexplored particularly of its filamentous fungal diversity. This present study examined the diversity and abundance of filamentous fungi, pH, moisture and organic matter contents as well as elemental analysis of the Plantation soils which have stood uncultivated for years. Soil samples were collected (at a depth of 0-10 cm) from three locations selected randomly with cleaned and surface sterilized soil auger. The soil samples were collected on a monthly basis for 12months during both dry and rainy seasons. A total of 669 positive isolations were obtained. Of the number, 439 isolations were mesophilic, 109 were thermotolerant and 121 were thermophilic isolates. A total of 387 isolations were obtained during the dry season and 282 during the rainy season. The dry season had a higher diversity index than the rainy season. The most dominant genus was *Aspergillus* with *A. fumigatus, A. niger, A. oryzae and A scleretiorum* the most abundant species. The genera of *Aspergillus* and *Penicillium* had the highest species richness. Fungal diversity and abundance were influenced by soil pH, moisture and organic matter content.

Keywords- Artemisia annua, soil, season, filamentous fungi, Nigeria.

I. Introduction

Soil has been observed as a complex ecosystem with physicochemical parameters that hold enormous number of living organisms. Microorganisms (bacteria, archaea, fungi, and protozoans) are very important in all processes related to soil function. Some of these processes include soil formation and soil structure. Microorganisms are also responsible for the mineralization process in ecosystems. They act on the organic matter to release CO_2 (cycling of carbon), nitrogen, phosphorous, and sulfur, which could be absorbed directly by plants. In addition, the microbial constituents of soil are entirely responsible for the degradation of toxic molecules (Olson *et al*., 2000; Nannipieri *et al*. 2003). Rigbelis and Nahas (2004) reported that the most important soil nutrient supply to the forest soil environment is the one derived from litter decomposition by actions of organisms under conditions of high air, temperature and soil moisture content. These organisms mobilized the chemical elements in the litter and make them re-absorbable by plant roots. They are able to perform these roles because of their ability to obtain nutrients through absorption. Soil fungi play an important role as major decomposers in the soil ecosystem. They play an obvious role in nutrient cycling by regulating soil biological activity (Arunachalam *et al*., 1997). The rate at which organic matter is decomposed by the microbes is correlated to the chemical composition of the substrate as well as environmental conditions. Many researchers have done a number of studies on the distribution of soil microfungi in Forest and agricultural fields. (Arunachalam *et al*., 1997; Carney and Matson, 2006; Kennedy *et al*., 2005; Chung *et al*., 2007).

The present study examined the diversity and abundance of filamentous fungi, pH, moisture and organic matter contents as well as elemental analysis of *A. annua* Plantation soils which have stood uncultivated for years in University of Jos, Nigeria.

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II. Materials and methods

A. Collection of soil samples and determination of Soil temperature and amount of rainfall

Soil samples were collected from three different locations in the *A. annua* plantation from a depth of 0-10cm. This was done using a clean and surface sterilized soil auger. The soil samples were packaged in well labeled clean cellophane bags and were taken to the laboratory for processing. The temperature of the soil at the three different locations was determined by the use of thermocouple. The thermocouple was inserted into the soil up to depth of 10cm and allowed to stay for 10minutes, after which the temperature reading was obtained and recorded.

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The readings for the amount of rainfall within the sampling period were collected from University of Jos metrological division.

B. Soil ecological and elemental analysis

The soil pH was measured by mixing the soil with water in the ratio of 1:5 and was determined by Jenway digital pH meter model 3310.

For the moisture content determination, 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.

For the organic matter content determination, soil samples (30 grams) previously dried to a constant weight in hot air oven set at 110^{o}C were used in the determination of the percentage (%) organic matter content. The soil samples were put in porcelain crucibles and the crucibles were placed in a muffle furnace and heated at 400^{o}C for 3hours. The samples were cooled and the percentage organic content of the soil samples was determined in triplicates and then recorded.

The concentrations of calcium (Ca), Potassium (K), magnesium (Mg) and Sodium (Na) were recorded as mg/kg of metal using atomic absorption spectrophotometer (BUCK 210 VGP). Nitrogen in the soil samples was determined by Kjeldahl method. The percentage nitrogen was then determined by distillation using 40% NaOH and 4% boric acid. It was then titrated against 0.01N HCl.

B. Isolation and identification of fungi from the different soil samples

Isolation of fungi from the soil samples were carried out by soil plate method (Warcup, 1950) using malt extract medium. The fungal isolates were identified with the help of existing keys (Thom and Raper 1945; Subramanian 1971; Samson *et al*., 1984).

C. Diversity of fungal Isolates between the dry and rainy seasons

The diversity of filamentous fungi in the dry and rainy seasons was compared using the Shannon-Wiener's index $H = -\Sigma$ PilnPi, where Pi is the frequency of fungal species occurring in a season. The % similarity of fungal species between seasons was calculated and compared using the Sorensen's similarity index (S) = C / (A+B+C) × 100 where, A = total number of species in dry season, B = total number of species in rainy season, C = total number of species in both seasons.

III. Results and discussion

A. Climatic conditions in the study area

The climatic conditions in Gangnum indicated that the highest temperatures ranging from 30-32^{o}C were from February to April. Rainfall within the ranges of 2-12mm increased from April to September and had the highest peak in July (12mm), with a reduction (8mm) in August. Temperature was also found to have increased approximately from October with highest peak in March 32^{o}C and then decreased as rainfall increased. The climatic conditions in Gangnum are illustrated in Figure 1. Factors such as temperature and humidity due to seasons have been known to affect fungal populations in a community (Lodge 1997; Kenney *et al*., 2006). In the present study (results not shown), *Chaetomium* and *Aspergillus* species as well as other hyphomycetes, thermotolerant and thermophilic species were isolated in abundance during dry season and had peaks in February-April before the rainy season began.

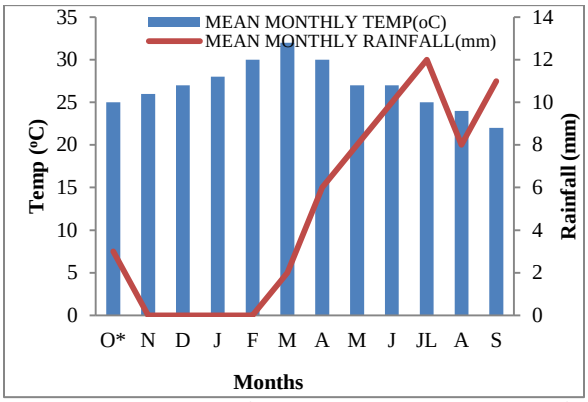

Fig. 1: Seasonal variations of mean monthly temperature and rainfall in Gangnum, Langtang South for the period of investigation

***O= Oct., N= Nov., D= Dec., J= Jan., F= Feb., M= Mar., A= Apr., M= May, J= Jun., JL= Jul., A= Aug. and S= Sept..**

B. Ecological and elemental analyses of the soil samples

The ecological analyses of the soil samples revealed a pH range of 5.80-7.21, moisture and organic matter content values ranging from 0.62-0.80 and 1.84- 4.30 respectively among the soil samples (Table 1). There

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was variation in the Nitrogen (N) concentration (0.012-0.21%), P (1.8- 3.5mg/kg), Na(1.1-2.4mg/kg), K(26-80mg/kg),Ca(800- 1072mg/kg), and Mg (74-98mg/kg) as shown in Table 1.

Table 1: Ecological and Elemental Parameters of the Soil Samples

Parameter	Soil Sample A*	Soil Sample B	Soil Sample C
Ph	5.80^{b}±0.071	6.65^{a}±0.071	7.21^{c}±0.071
Moisture %	0.72^{b}±0.072	0.62^{a}±0.072	0.80^{b}±0.072
Organic Matter %	4.30^{c}±0.058	3.67^{a}±0.058	1.84^{a}±0.058
Nitrogen (N)%	0.099	0.21	0.012
Phosphorus (P) mg/kg	2.3	3.5	1.8
Sodium (Na) mg/kg	1.1	1.2	2.4
Potassium (K) mg/kg	32	80	26
Calcium (Ca) mg/kg	988	1072	800
Magnesium (Mg) mg/kg	74	98	88

*A, B and C=Soil samples from locations A, B, C. Mean having the same superscripts in the same column are not significantly different from each other at 5% probability level.

C. Isolation and diversity of fungal isolates

A total of 669 fungal isolates were obtained from the study, 387 in the dry season and 282 in the rainy season. The isolates represented many fungal groups such as phycomycetes including aquatic phycomycetes, hyphomycetes including ascomycetes, thermophilic and thermotolerant species. The isolated ascomycetes included *Chaetomium. Aspergillus, Fusarium, Penicillium, Trichoderma* species and others. The genus *Aspergillus* was recorded as the most dominant genera (Table 2). This result is in line with the findings of Cavalcanti *et al*. (2006) and Nilima Wahegaonkar *et al.* (2011).

The overall diversity of fungal species in the *Artemisia annua* plantation was high according to the Shannon - Wiener index (Table 2), however, the dry period had higher fungal diversity (387) than the rainy season (282) as was determined with Sorensen's similarity index. The diversity of fungi during dry season between February- April was high in species richness for *Aspergillus*, other hyphomycetes, thermotolerant and thermophilic species and had a higher diversity index than the species isolated in rainy season. These groups of fungal species have been reported by Nicot (1960) to be strongly pigmented and that such pigmentation could be protecting them against strong light and desiccation. Similar results have been reported (Kodseub, 2007).

Table 2: Diversity Index of Isolated Fungi from *Artemisia annua* Plantation Soil

	Species	Total no of isolations	Σ (P) (Proportion)	Σ(Lnp)	Shannon-Weiner species diversity index Σ(p Ln p)
1	Aquatic phycomycetes	8.000	1.000	-1.451	-0.662
2	Chaetomium	43.000	1.000	-14.199	-1.872
3	Aspergilli	118.000	1.000	-45.762	-2.689
4	Fusaria	49.000	1.000	-16.861	-2.053
5	Phycomycetes	50.000	1.000	-23.554	-2.249
6	Penicillia	57.000	1.000	-27.732	-2.278
7	Trichoderma	27.000	1.000	-5.729	-1.344
8	Other Hyphomycetes	87.000	1.000	-30.127	-2.461
9	Thermotolerant *sp*	109.000	1.000	-29.999	-2.470
10	Thermophilic species	121.000	1.000	-52.276	-2.878

IV. Conclusion and ongoing work

The results of this study revealed that there was variation in the population and diversity of fungi in the soil samples obtained from different locations of *A. annua* Plantation soils. The pH values obtained from all the soil samples favoured microbial activities. The work also established that there was a great seasonal effect on the population of filamentous fungi isolated from the plantation soils. Further analysis is to be performed on how the effects of the activities of the fungal species on organic matter decomposition and humus formation would enhanced the nutrient content of the plantation soil for improved agricultural activity.

V. Acknowledgement

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The authors are thankful to the Management of University of Jos and Tertiary Education Trust funds (TETFUNDS) for their sponsorship. Authors are also grateful to F. B. Falemare for helping in the statistical analysis of the data.

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