Albanian j. agric. sci. 2017;16 (4): 143-150 *Agricultural University of Tirana*

RESEARCH ARTICLE **(Open Access)**

Occurrence of Fungal Strains with Herbicidal Potentials in Agricultural Soils of Southern Guinea Savanna Agro - ecology of Nigeria

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Abstract

In a laboratory experiment, herbicidal potentials of fungal strains in agricultural soils were investigated. This was with the view of using them to compliment synthetic herbicides which have been associated with adverse effects on the soil ecosystem and thus reduce environmental pollutions while controlling weeds. Soils were collected from four selected farm sites within the Southern Guinea Savanna (SGS) agro ecological zone of Nigeria. Fungi were isolated using the soil dilution method and were identified using their colony and microscopic morphological characteristics. They were screened for their herbicidal properties on the foliages of *Amaranthus hybridus* using leaf necrosis assay. The percentage frequencies of occurrence of twenty-one fungal isolates were determined. The predominant fungal strains were *Aspergillus sydowii* (43.47%) and *Alternaria alternata* (30.43%), *Aspergillus tereus* (53.85%) and *Aspergillus ustus* (23.08%), *Aspergillus niger* (44.44%), *Aspergillus tereus* (33.33%), *Penicillium marneffei* (58.06%) and *Fusarium vertilliodes* (25.81%) in soils from Kogi, Kwara, Niger and Benue farm sites respectively. A very high percentage (80-85%) of all the fungal isolates from the soils showed herbicidal properties. The isolated fungal strains had varying degrees of herbicidal properties. Two fungal strains, *Aspergillus fumigatus* and *Penicillium citrinum* had the highest strengths as biocontrol agents as indicated by the large necrotic lesions produced on the foliages of *Amaranthus hybridus* It was concluded that these two have the potentials for use as bioherbicides. Further investigations were recommended to study the secondary metabolites produced by these fungi and their effects on selected weeds.

Keywords: Bio-herbicide, Soil Fungi, Leaf necrosis assay.

1. Introduction

Agricultural soil is made up of mineral particles and organic remains and serves as the medium for growth and development of crops. The properties of the soil influence its composition and microbial activities. Soil is one of nature's most complex ecosystems and one of the most diverse habitats on earth: it contains a myriad of different organisms, which interact and contribute to the global cycles that make all life possible [6]. Nowhere in nature are species so densely packed as in soil communities; however, this biodiversity is little known as it is underground and largely invisible to the human eye [7]. Several microbial functions can provide important services to man either in form of supporting services that are not directly used by humans but which are essential for providing all other services. These include nutrient cycling and formation of soil. Also, composition of the atmosphere, environmental pollution, pest and disease incidence in ecosystems, and human diseases are all influenced by soil biodiversity. Moreover, soil organisms also contribute to providing services that directly benefit people, for example the genetic resources of soil microorganisms can be used for developing novel pharmaceuticals [6]. Biological control refers to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations. Invasive weeds continue to threaten the productivity of agricultural lands and natural areas; however, for many weeds adequate, cost-effective control measures presently are not available [13]. Fungi are the trusted source of structurally complex and pharmacologically important secondary metabolites [23]. Use of biological herbicides requires a shift in thinking from the use of chemical herbicides, as biological controls will most likely not eliminate a weed problem as quickly or as thoroughly as some herbicides on an annual basis; the goal is to inhibit the weed pest below

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(Accepted for publication October 20, 2017)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

an economically damaging threshold over a long time period in order for beneficial species to gain a competitive advantage [9]. There are a number of reasons for developing microbial herbicides, and those include the potential for herbicide resistant weeds; chemical herbicides may persist in soil for longer than one growing cycle reducing the productivity of the soil, thus limiting options for crop rotations; there is the potential for fewer undesirable effects to the environment than from chemical herbicides; and finally there is the potential for injury to non-target organisms [10].There is a vast diversity of microorganisms in our environment whose potentials have not been unearthed. The discovery and characterization of such microorganisms will make available alternatives or complements to chemical herbicides for weed control, thus reducing degradation due to tillage and use of inorganic compounds. Therefore, this paper investigates the occurrence of fungi with herbicidal properties in soils of Southern Guinea savanna agro-ecological zone of Nigeria.

2. Materials and Methods

Collection and pretreatment of soil samples

Soil samples classified as alfisols were collected from the top soil of four different farm sites from southern Guinea savanna of Nigeria (Kwara 7° 33´N 04° 33´E, Kogi 7° 33´N 06° 14´E, Niger 10° 19´N 04° 36´E and Benue States 7° 43´ 50´´N 08° 32´10´´E). The soil samples were collected randomly within the top 15cm of the soil with the aid of soil auger and were transported to the laboratory in sterile zipper polyethylene bags. Soil samples from each of the farm sites were composited, air-dried and sieved through 2mm sieve.

Determination of some physiochemical properties of the soil

The Walkley-Black method as described by [12] was used in the determination of percent organic carbon and organic matter; pH of soil sample in water and in 1N KCl (1:2.5) were as described by [3]; moisture content determination was by weight loss on ignition method; total nitrogen determination was by microKjeldahl distillation method [1] and Bray 1 method [4] was used for determination of available phosphorus in soil.

Isolation of fungal strains

Serial dilution agar plating method [16] was adopted. Ten-fold serial dilutions of the soil suspension from each soil sample were carried out in sterile water. Sterilized Potato Dextrose Agar (PDA) plates were then inoculated with 10^{-3} dilutions of soil samples and incubated for ten (10) days at 28°C. Growths of the different fungal strains were observed after the incubation period. Pure cultures of isolated strains were made on PDA medium and stock cultures were prepared on PDA slants.

Screening of fungal isolates for herbicidal potentials

Leaves of *Amaranthus* plants used for herbicidal assay were collected from the University of Ilorin Teaching and Research farm. Fresh leaves were collected in sterile polythene bags, kept in ice box, and transported to the laboratory immediately. Leaf necrosis assay was carried out with aliquots (10, 100 and 1000 µg/ml) of concentrated extracts of all isolated fungal conidia as described by [20]. The expanded leaves were surface sterilized with ethanol and washed with sterile distilled water to remove ethanol from the surface before inoculation with fungal spores. The inoculated leaves were then transferred to petri dishes containing moistened cotton balls and filter papers. The plates were incubated at room temperature for 2 days. The inoculated leaves with respective extracts were observed for the development of necrotic lesions. The fungal isolates that caused necrotic lesions on detached leaves were identified and characterized.

Production of secondary metabolites from fungal strains

Czapex Yeast Broth (CYB) {*Oxoid*} composed of sucrose (30.0g), $NaNO_3$ (2.0g), Magnesium glycerophosphate (0.5g), KCl (0.5g), $FeSO_4$ (0.01g), K_2SO_4 (0.35g) and 1 litre H_2O was used as culture medium for production of secondary metabolites fungal isolates as described by [20]. The medium was autoclaved at 121 ºC, 15 *P*si for 15 min, and poured in Petri dishes. The fungal spore suspensions derived from 7 days old slant culture of those fungal strains that gave necrotic lesions on *Amaranthus* leaves were inoculated in separate flasks containing the CYB medium at rate of 0.1ml spore suspension per 20 ml medium. Fungal spore suspensions derived from 7 days old slant cultures of the fungal strains that gave necrotic lesions on *Amaranthus* leaves were inoculated in separate flasks containing the medium at rate of 0.1ml spore suspension per 20 ml medium.

Extraction of fungal metabolites from liquid medium

At the end of the incubation period, 250µl of 40% HCl was added to each of the incubated flasks to separate the media components. The fungal mycelia were ground using electric blender and equal volumes of ethyl acetate were added to each flask. The contents in each of flasks were mixed for 40 minutes and the mycelia filtered with Whatman filter paper No. 1. The filtrates were transferred into separating funnel so as to separate the organic layer containing the metabolites and then washed with 2M brine solution to remove the impurities. Anhydrous Na_2SO_4 was used to dehydrate the organic layer which was again filtered and the recovered organic layers containing the crude metabolites were concentrated on rotary evaporator at 45^{o}C. The concentrated extracts were dissolved in sterile di-methyl sulfoxide (DMSO) and used to assay for herbicidal activity by observing the development of necrotic lesions on leaves of *Amaranthus hybridus*.

Identification and characterization of fungal isolates with herbicidal potentials

Cultural characteristics of fungal isolates such as structure of hyphae, arrangement of spores and pigmentation of the colonies were observed on PDA plates. Purified fungal strains were observed under light microscope (× 40 magnification) for microscopic morphology. The fungal mycelia were stained with cotton blue in lactophenol stain on slides. The type of asexual spores; sexual reproductive structures and presence or absence of septa in hyphal filaments were observed and recorded. Identification of isolates was then made with reference to standard texts.

Data analysis

The percentage frequencies of occurrence of the soil fungi encountered were determined.

3. Results and Discussion

Characterization of the fungal isolates from Agricultural soils

Figures 1, 2, 3 and 4 show the percentage frequencies of occurrence of the fungal isolates with herbicidal properties from soils from the different farm sites. *Aspergillus sydowii* predominates the soil from Kogi farm site (43.47%) followed closely by *Alternaria alternata* (30.43%) and organisms with the lowest frequency of occurrence were *Aspergillus fumigatus*, *Aspergillus glaucus* and *Aspergillus flavus* (2.17% each). In the soil from Kwara farm site, *Aspergillus terreus* had the highest percentage of occurrence with 53.85% of the total isolates. *Aspergillus ustus* followed with 23.08% with *Aspergillus niger and Penicillium sp.* having the lowest (7.69%). *Aspergillus niger* (44.44%) predominated the soil from Niger state followed by *Aspergillus terreus* and *Microsporium canis* with 22.22% and 33.33% respectively. *Fusarium verticillioides* (58.06%) predominated the soil from Benue farm site followed by *Penicillium marneffei* (25.81%) with *Aspergillus niger* (3.23%) having the least percentage frequency of occurrence. The species from the genera *Aspergillus, Penicillium and Fusarium* were observed to be the most dominant in the soils from the four selected farm sites. The dominance of the *Aspergillus species* and *Penicillium species* in the soils as revealed in the results could be attributed to their ability to produce large number of asexual spores. They have been reported as predominant fungi in many soils [5]. The number of soil fungal strains isolated from each of the soil samples collected from the different farm sites were Eight (8) in Kogi, Five (5) in Kwara, Three (3) in Niger and Five (5) in Benue. The lowest number of strains isolated was obtained in Niger soil as shown in Figure 2. This could be partly due to the soil pH (6.0-6.4) which is slightly higher than those of other farm sites. Fungi are more prevalent in soils with pH within 4.5 to 5.5 [2].

The variations in the number of fungal isolates from the different soils might be due to the variation in soil properties and climatic factors. Table 1 shows some physicochemical properties of soils from the four farm sites used for the study. The percentage moisture content of soils from Kogi (21.16%) and Benue (20.20%) farm sites were observed to be the highest while that of Niger farm site (17.67%) was the lowest for the selected farm sites studied. This might be responsible for the higher number of isolates from Kogi and Benue farm sites as microorganisms require adequate

moisture for their physiological activities. Reduced microbial activities are associated with dry soils. Tichich *et al.* [22] found that for optimum population growth of the pathogen *Pseudomonas syringae* pv. *tagetis* targeting Canada thistle (*Cirsium arvense*), periods of wet weather were required.

Table 1. Physico-chemical properties of soil from the different farm sites within the southern Guinea savanna agro-ecological zone of Nigeria

Sources of soil samples	% Organic Matter	% Organic Carbon	pH Water	pH KCl	% Nitrogen	Available Phosphorus (ppm)	% Moisture content
S1	0.97	0.56	5.7	5.3	0.48	7.54	20.69
S2	0.93	0.54	6.4	6.0	0.34	8.60	17.67
S3	0.69	0.40	5.4	5.0	0.50	4.42	20.20
S4	0.52	0.30	5.5	5.1	1.15	7.57	21.16

Keys
S1= Soil sample collected from Benue farm site
S2= Soil sample collected from in Niger farm site
S3= Soil sample collected from Kwara farm site
S4= Soil sample collected from Kogi farm site

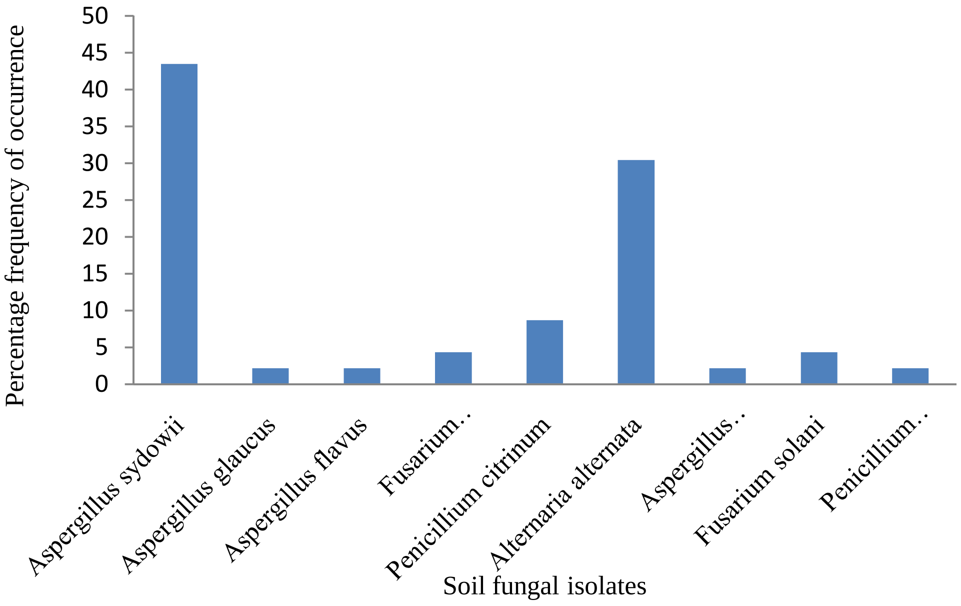

Figure 1. Occurrence of fungi in Kogi farm site

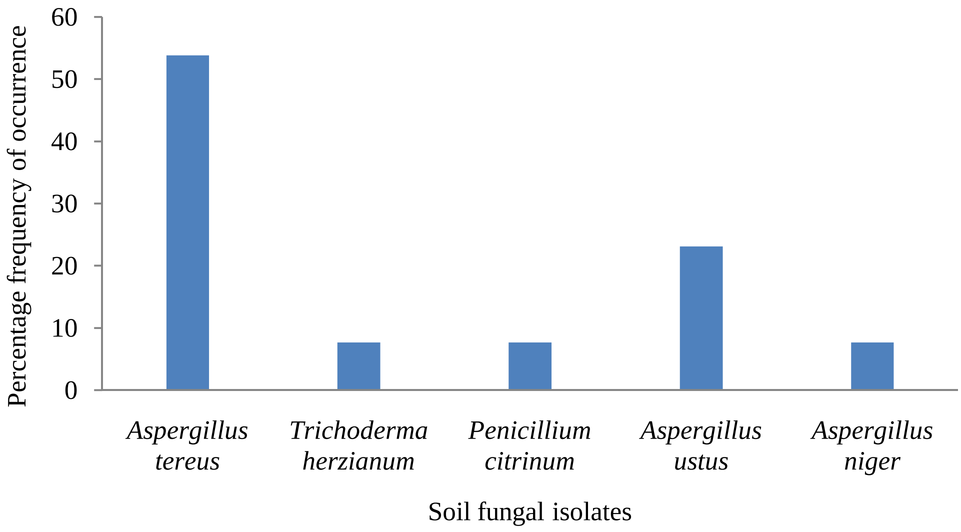

Figure 2. Occurrence of fungi in Kwara farm site

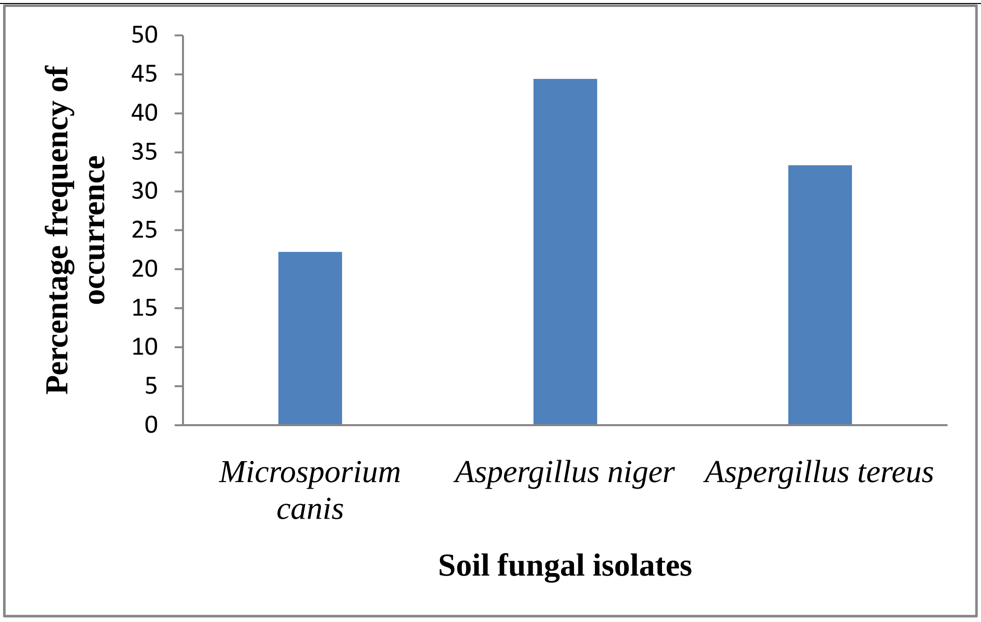

Figure 3. Occurrence of fungi in Niger farm site

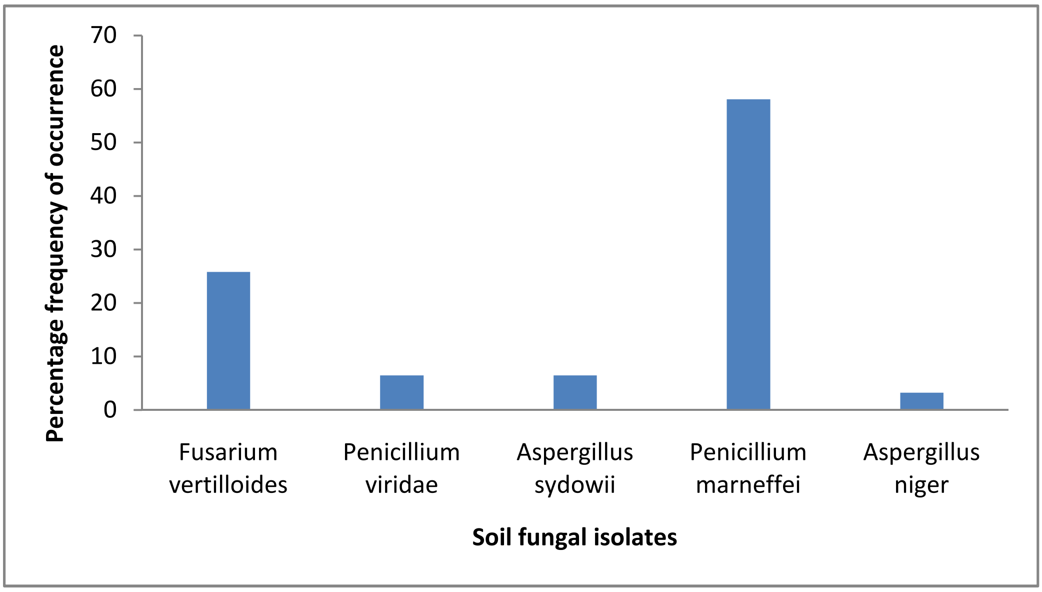

Figure 4. Occurrence of fungi in Benue farm site

Moisture requirements often limits candidates for biocontrol. Favorable moisture and temperature conditions are critical to the efficacy of many mycoherbicides.

According to Osundare [17] majority of Alfisols of the Southern Guinea Savanna are mostly within the sandy-loam textural class and their fertility level is usually low creating a need for constant addition of organic or inorganic fertilizers. The fertility levels of the soils used for the study were relatively high probably because they were collected from fields cropped with legumes and maize. Legumes have the capacity to fix Nitrogen to the soil. The high fertility levels can also be as a result of fertilizer applications in all the four farm sites.

The physicochemical properties of soil and its nutrients status influence the microbial population both quantitatively and qualitatively because soil microorganisms just like higher plants depend entirely on soil for their nutrition, growth and activity [19].

Screening of fungal isolates for herbicidal potentials

Table 2 shows the herbicidal strengths of the fungal isolates from the soils of the different farm sites. Out of the fifteen (15) fungal isolates that were selected for the screening, four (4) showed greater potentials to be used as biocontrol agents against the foliage of *Amaranthus hybridus* Linn. by producing large necrotic lesions on the foliage especially at the highest concentrations (1000 µg/ml) used for the study. The effect of the crude extracts of some fungal isolates on the foliage of *Amaranthus hybridus* are shown in Plate 1. The four fungal isolates in order of their ability to produce necrotic lesions on foliage of *Amaranthus hybridus* are *Aspergillus fumigatus* > *Penicillium citrinum.* > *Penicillium viride* > *Fusarium oxysporium*.

Table 2. The herbicidal strengths of the fungal isolates from soils of four farm sites within the southern Guinea savanna, Nigeria

Identities of the soil Fungal Isolates	Herbicidal strengths of different concentrations of crude extracts of soil fungi 10µg/ml	100µg/ml	1000µg/ml
Fusarium oxysporium	++	+++	+++
Aspergillus glaucus	+	+	+
Aspergillus fumigatus	+++	+++	+++
Fusarium verticilloides	+	++	+
Penicillium citrinum	+++	+++	+++
Alternaria alternate	+	+	+
Aspergillus niger	++	++	++
Fusarium sp.	+	+	+
Aspergillus tereus	+	+	++
Trichoderma harizanum	+	+++	++
Aspergillus ustus	+	++	++
Microsporum canis	++	+	+
Penicillium marneffei	+	++	++
Penicillium viridae	++	+++	++
Fusarium solani	++	++	+
Control	-	-	-

Keys
+++: High herbicidal strength (large necrotic lesions)
++: Moderate herbicidal strength (medium sized necrotic lesions)
+: Low herbicidal strength (small sized necrotic lesions)
-: No herbicidal strength (no necrotic lesions)

Fungi are the trusted source of structurally complex and pharmacologically important secondary metabolites [23]. *Aspergillus fumigatus* and *Penicillium citrinum* which gave the largest necrotic lesions on the foliage of *Amaranthus hybridus*, an indication of their herbicidal potentials were selected for futher study. Khatta *et al.* [14] tested the crude metabolites of *Penicillium* and *Aspergillus* species against *Lemna* for phytotoxicity and reported positive activities. Several workers have affirmed that the two genera have members that are producers of bioactive secondary metabolites like polyketides, terpenoids, xanthones etc which have been shown to have antibacterial, antifungal and cytotoxic properties [12, 9, 21, 11].

Plate 1. Effects of crude extracts from *Penicillium citrinum* on the foliage of *Amaranthus hybridus*

Key:
10, 100, 1000: Different concentrations of the crude extracts (µg/ml)
KO19: *Penicillium citrinum*

4. Conclusion

This study has revealed that a high percentage (80-85%) of fungal isolates from SGS soils have herbicidal properties. However, *Aspergillus fumigatus* and *Penicillium citrinum* have relatively higher bio herbicidal potentials than others. The secondary metabolites produced by these fungi which could control weeds would be tested in further studies.

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