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Research Article

BIODEGRADATION OF GLYPHOSATE CONTAINING HERBICIDE BY SOIL FUNGI

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ABSTRACT

Ten fungal isolates were isolated from two herbicide-contaminated soil farms obtained from Amoyo and the University of Ilorin environment in Kwara State after enrichment with mineral salt medium (MSM) supplemented with glyphosate-containing herbicide. The growth of fungal isolates was efficiently stimulated by the organophosphorus herbicide. Fungi isolated were subjected to screening by varying the herbicide concentrations from 0.1 to 3%, which is prepared with MSM. This screening showed that all the fungal isolates had the ability to act in the biodegradation process. However, varying degradative potentials were observed, as some had heavy growth while others had only slight or no growth as the concentrations of the herbicide increased. The ten fungal isolates were characterized and identified as *Aspergillus niger*, *Penicillum spinulosum*, *Aspergillus terrus*, *Aspergillus flavus*, *Fusarium oxysporum*, *Mucor* spp., *Aspergillus oryzae*, *Aspergillus tamari*, *Rhizopus stolonifer,* and *Trichoderma koningii*, and these were reduced to six after screening with 3% concentration of the herbicide. Four isolates (*A. niger*, *F. oxysporum*, *Mucor* spp., and *A. flavus*) were selected based on their growth ability on the medium during screening and were used in the biodegradation study. However, there is an increase in fungal dry weights ranging from 8.60 to 18% for 12 days. This shows that these fungi can be employed in the biodegradation of herbicides since they are potentially effective species and are environmentally safer alternative to protect the soil from the contamination of glyphosate-containing herbicide residues.

Keywords: Fungi, Isolates, Herbicide, Glyphosate, Biodegradation.

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INTRODUCTION

Organophosphorus pesticides are actually more widely used in the world; these pesticides affect the nervous systems of insects and humans, in addition to influencing the reproductive system [1,2]. These chemical agents block prolonged inhibition of the activity of the enzyme cholinesterase, responsible for the nervous impulse in organisms [3]. The excessive use of organophosphorus in agriculture has originated serious problems in the environment [4]. Although these pesticides degrade quickly in water, there is always the possibility that residues and by-products will remain at relatively harmful levels in organisms [5].

Several biological techniques involving the biodegradation of organic compounds by microorganisms have been developed and are still being developed [6]. The use of microorganisms, either naturally occurring or introduced, to degrade pollutants is called bioremediation [7]. In addition, they are robust organisms and are generally more tolerant to high concentrations of polluting chemicals, such as some bacteria [8]. However, the aim of this study is to exploit the ability of fungi to degrade herbicide residue from agricultural soils.

METHODS

Sterilization of glassware and other materials

All glassware used was thoroughly washed with detergent, rinsed, and allowed to dry. The glassware was then wrapped with aluminum foil and sterilized in the hot air oven at 170°C for 60 min. The distilled water used for serial dilution was autoclaved at 121°C for 15 min. The work bench was swabbed with 70% alcohol before and after every experiment.

Sample site

Two agricultural sites contaminated with herbicide were selected: these were farms from Amoyo in Ifelodun Local Government Area of Kwara State and the Nursery Section of the Department of Forestry, University of Ilorin, Kwara State. At each location, four different samples were collected.

Soil sampling

Two soil samples were collected at four different points at each location and were labeled A1, A2, A3, A4, and B1, B2, B3, and B4, respectively. At each point, soil samples were collected randomly 5–10 cm beneath the surface of the soil using a sterile hand trowel and packed in sterile polythene bags properly labeled. The samples were immediately transferred to a microbiology laboratory for analysis.

Herbicide

Two brands of herbicides containing one active ingredient, glyphosate, were used in this study. The brands were Force Up and Golden Sate with the active ingredient of analytical standard glyphosate (44.1%), which was provided by an agrochemical shop in Ilorin metropolis, Kwara state.

Physicochemical analysis of soil samples

The physicochemical characteristics of the soil samples that were determined include temperature, pH, percentage moisture content, and organic matter content. Soil pH, percentage moisture content, and organic matter content were determined based on the method described by Pramer and Schmidt [9].

Preparation of mineral salt agar (MSA) medium

Preparation of stock mineral salt medium for fungi

Stocks were prepared inside plastic bottles with sterile distilled water using the following composition of mineral salts as described by Ashour *et al.* [10]. The constituents of fungi are presented in Table 1.

Table 1: Composition of mineral salt agar

Fungi (L)	For 150 mL
2.0 g $NaNO_3$	15 mL of 2% $NaNO_3$
1.0 g KH_2PO_4	7.5 mL of 2% KH_2PO_4
0.5 g $MgSO_4$	3.75 mL of 2% $MgSO_4$
0.5 g KCl	3.75 mL of 2% KCl
0.01 g $FeSO_4.7H_2O$	0.08 mL of 2% $FeSO_4.7H_2O$
Herbicide	1/2/3 mL of forceup/goldensate
15 g Agar-agar	2.3 g of Agar powder

Preparation of media

All these mineral salts were measured into different plastic bottles and dissolved with 100 mL of distilled water as stock. Calculated volumes of each mineral salt were taken from various stocks with sterile pipettes. In order to make a 150 mL solution, 100 mL of distilled water and 20 mL of tap water were added as sources of trace elements to make up the 150 mL, followed by 2.3 g of agar-agar. The mixture was heated, homogenized, and autoclaved at 121°C for 15 min. The volume of each stock solution used for 150 mL of MSA medium for fungi and bacteria is described earlier above.

Microbiological analysis of soil samples

Isolation of fungi from soil samples

The spread plate method was used to isolate fungi from soil samples [11]. Fungi growths were examined after incubation at room temperature for 48–72 h, following the method described by Dubey and Maheshwari [12].

Screening of fungal isolates

Screening of fungal isolates and growth capability on the herbicide MSAmedium was done qualitatively following the method described by Ashour *et al.* [10]. Fungal screening was carried out by monitoring the growth capability of glyphosate-containing media at different concentrations (0.1%, 1%, 2%, and 3%).

Characterization and identification of fungal isolates

The most efficient herbicides for degrading fungal genera were examined macroscopically and microscopically to determine their macroscopic and morphological characteristics, respectively. The isolates were identified according to the keys of Onions *et al*. [13]. Selected fungal isolates were used for the biodegradation experiment.

Fungal biodegradation study

The composition of the mineral salt broth medium used for the fungal degradation study was the same composition of stock mineral salt medium for fungi, with the exemption of agar-agar. Four hundred milliliters of the medium were prepared in a 500 mL conical flask, and 6 ml of the test herbicide was added as a source of carbon and energy before the medium was sterilized in an autoclave at 121ºC for 15 min. The progress of the degradation study was assessed by measuring the change in pH and percentage of dry weight over the period of time. Changes in pH of the mineral salt broth were monitored, and the percentage of dry weight was determined quantitatively in the course of the biodegradation period based on the method described by Ekundayo and Osunla [14].

RESULTS

Isolation and characterization of fungal isolates

A total of seven fungal isolates grew on MSA supplemented with 3% (w/w) Force Up herbicide. Their growth was taken as indicative of their biodegradative ability. *Fusarium oxysporum* showed the weakest growth, while *Aspergillus niger* showed the heaviest in terms of the diameter of colony, on MSA. However, the best four fungi, *A. niger*, *Aspergillus flavus*, *F. oxysporum*, and *Mucor* spp., were used for the fungal biodegradation study. Details of the degree of growth of all fungal isolates are shown in Table 2.

Table 2: Growth of fungal isolates in mineral salt agar medium supplemented with 2% and 3% (v/v) of force up and goldensate herbicides brands

S. No	Fungal isolates	Degree of growth in 2% MSA	Degree of growth in 3% MSA
	Force up		
1.	*Aspergillus niger*	++++	++++
2.	*Aspergillus flavus*	++++	++++
3.	*Mucor* spp.	++++	+++
4.	*Fusarium oxysporum*	++++	+++
5.	*Aspergillus oryzae*	++	-
6.	*Trichoderma koningii*	+	-
7.	*Aspergillus tamari*	+++	++
8.	*Rhizopus stolonifera*	++	-
9.	*Penicillium spinulosum*	+++	++
10.	*Aspergillus terrus*	+++	+++

MSA: Mineral salt agar medium. +++++: Profused growth, ++++: Heavy growth, +++: Moderate growth, ++: Slight growth, +: Faint growth, -: No growth

Description of fungal isolates

Isolates F1, F2, F3, and F4 were shown and described in Plates 1-4.

Isolate F1: It appeared as whitish colonies that turned black due to the formation of black conidia, with colonies spreading rapidly. Under microscopic examination, the hyphae were septate and profusely branched. Conidia were bonded in chains at the hips of sterigmata. Conidial heads are globose. The conidiophores were long, smooth, and hyaline (colorless). It was identified as *A. niger* (Plate 1).

Isolate F2: Bright to yellow, green colonies have the same color of green with a tinge of yellow on the reverse side of the plate. Conidiophores are coarsely rough; heads vary in size; loosely radiate; and phialides are borne directly on the vesicle. Conidia are globose. It was identified as *A. flavus* (Plate 2).

Isolate F3: Colonies are grey and of loose texture. Sporangiophores are branched, and sporangia are globose and small. Presence of chlamydospores in the sporangiophores. It was identified as *Mucus* spp. (Plate 3).

Isolate F4: The colony has thick white mycelium and a white color at the bottom of the plate; microconidia are oval and produced on simple short phialides. Macroconidia are septate and chlamydospores are present. It was identified as *F. oxysporum* (Plate 4).

Screening of fungal isolates in MSA

A. niger, *A. flavus*, *Mucor* spp., and *F. oxysporum* grew heavily. *Aspergillus tamari*, *Penicillium spinulosum,* and *Aspergillus terrus* grew moderately. *A. oryzae* and *Trichoderma koningi* grew slightly on MSA plates at 2% concentration of Force Up herbicide.

However, *A. niger* and *A. flavus* grew heavily on MSA plates, while *Mucor* spp., *F. oxysporum,* and *A. terrus* grew moderately at 3% on MSA plates. *A. tamari* and *P. spinulosum* grew slightly, while *A. oryzae*, *T. koningii,* and *Rhizopus stolonifer* showed no growth at 3% after a week. Details of the growth of all biodegrading fungal isolates on MSA plates are shown in Table 2.

Fungal biodegradation studies

Only four fungal isolates were selected and used for biodegradation, namely, *A. niger*, *A. flavus*, *Mucor* spp., and *F. oxysporum,* among all the fungi isolated from herbicide-contaminated soil were used for the biodegradative study of fungi. Parameters measured during the course of biodegradation were changes in pH and dried weight after 12 days.

Changes in the pH of mineral salt broth (MSB) during biodegradation

The initial pH of the MSB was 6.80; values of pH recorded from day 3 to day 12 include the following: *For A. niger,* the pH ranged from 6.60

to 6.90; this increases directly with the number of days; for *A. flavus,* the pH ranged from 6.70 to 6.90; this also increases directly with the number of days; for *Mucor* spp., the pH ranged from 4.40 to 5.30; there was a sharp decrease in the pH to 4.40 before later increasing gradually towards neutrality; and for *F. oxysporum,* the pH ranged from 4.50 to 4.80; this was similar with Mucor spp. Details of the changes in pH are shown in Fig. 1.

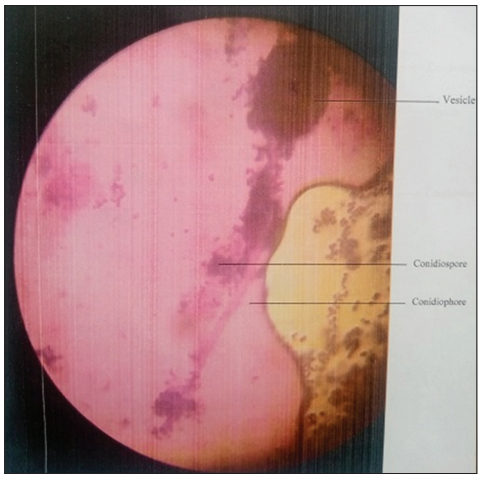

Plate 1: *Aspergillus niger* ×400

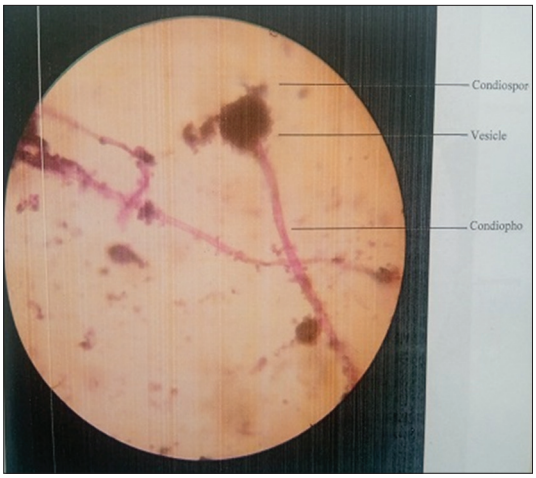

Plate 2: *Aspergillus flavus* ×400

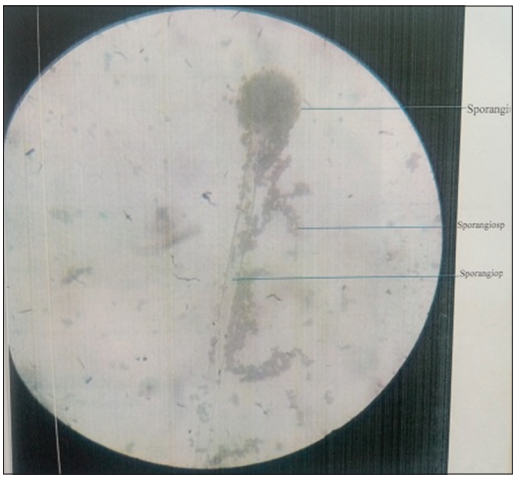

Plate 3: *Mucor* spp. ×400

Percentage of dry weight

The dry weight of the fungal isolates for the two brands of herbicide used was 18% for *A. niger*, 9.90% for *A. flavus*, 8.90% for *Mucor* spp., and 8.60% for *F. oxysporum*. Details of the dry weight are shown in Fig. 2.

DISCUSSION

Microbial analysis of herbicide-contaminated soils yielded viable fungi for all fungal isolates. Fungal growth at concentrations ranging from 0.1 to 3% herbicide supplement medium. These fungal isolates are known to be indigenous soil flora.

Ten (10) fungi isolates, namely: *A. niger*, *P. spinulosum*, *A. terrus*, *A. flavus*, *F. oxysporum*, *Mucor* spp., *A. oryzae*, *A. tamari*, *R. stolonifer,*

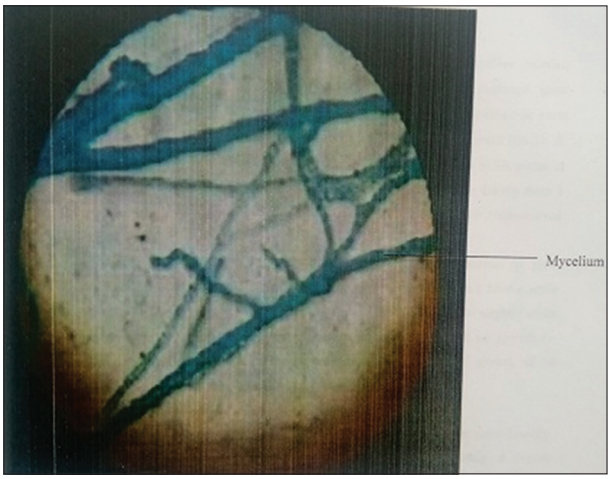

Plate 4: *Fusarium oxysporum* ×400

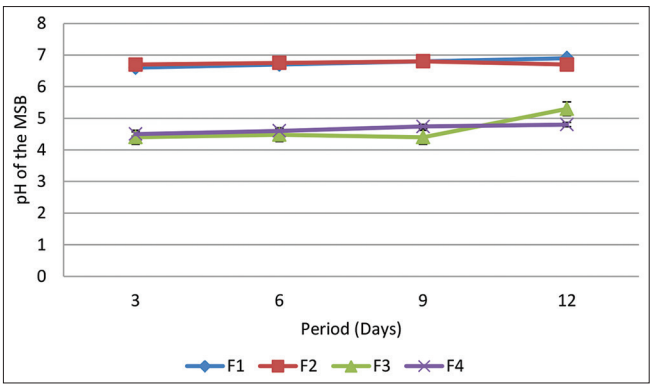

Fig. 1: Changes in pH of Mineral Salts Broth seeded with fungal isolates during fungal biodegradation

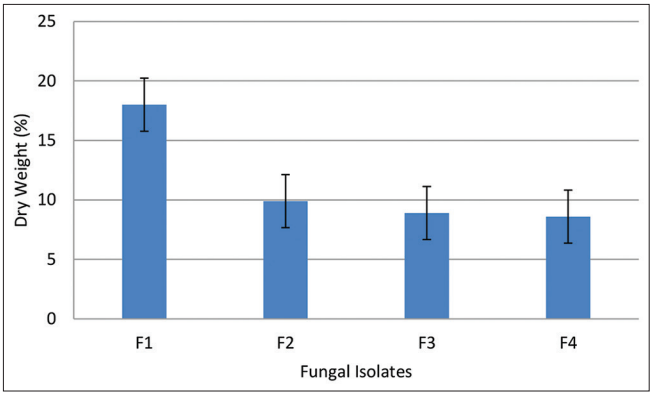

Fig. 2: Dry weight of fungal isolates at the end of biodegradation

and *T. koningii,* were isolated. *Aspergillus* spp. was the only common genus isolated. Aspergilli groups are commonly found in soil and decaying organic materials by converting resistant organic chemicals such as pesticides and herbicides into simplified metabolites and eventually into soluble benefits molecules. Fungi such as *Aspergillus* spp. play an important role in carbon cycling [15]. In this study, five species of *Aspergillus* were isolated. This observation was in agreement with Abdel-Hafez [16], who found the most frequent fungal genus, *Aspergillus*, in forty soil samples collected from desert soils in Saudi Arabia. Ashour *et al.* [10] also reported the isolation of 45 fungal isolates identical to the ten fungi isolated in this study. Similarly, these data were in agreement with Wardle and Parkinson [17], who studied the side effects of glyphosate on fungal species by applying a range of glyphosate concentrations (0, 2, 20, and 200 ppm herbicide). They found out that there were few fungal responses to glyphosate incubated in pure culture, where *Mucor hiemalis*, *F. oxysporum,* and *Penicillium nigricans* were largely unaffected by glyphosate at any concentration. *Mortierella alpine*, *Trichoderma harzianum,* and *Arthrinium sphaerospermum* were all significantly stimulated by 200 µg/g of glyphosate-containing herbicide. *Cladosporium cladosporioides* was significantly inhibited by higher glyphosate concentrations.

The increased use of herbicides in agricultural soils causes contamination of the soil with toxic chemicals. When herbicides are applied, the possibility exists that these chemicals may exert certain effects on non-target organisms, including soil microorganisms [18,19]. The microbial biomass plays an important role in the soil ecosystem, where it fulfills a critical role in nutrient cycling and decomposition [20]. When an herbicide is added to a cultivated medium, the various microorganisms may have different types of responses. Some microorganisms become intoxicated and lyse. Other microorganisms are resistant and tolerant to a pollutant and can increase their numbers and biomass because of decreased competition. Specific microorganisms will actually grow on organic pollutants.

This degradation pathway of glyphosate produces the major metabolite aminomethylphosphonic acid and ultimately leads to the production of water, carbon dioxide, and phosphate [21]. In the environment, Wardle and Parkinson [17,18] observed that the presence of glyphosate in the soil and the overall microbial activity of the soil, although the number of fungi and actinomycetes was not affected. In fact, studying the effect of glyphosate on the number of microorganisms in soil, microbial biomass, and soil respiration, Statton and Stewart [22] observed only a small increase in microbial biomass but no negative or positive effects with respect to the number of microorganisms or soil respiration. Haney *et al.* [23] and Busse *et al.* [24] evaluated the effect of glyphosate on the microbial community of soils and observed that microbial activity was stimulated in the presence of this herbicide. Glyphosate can stimulate the growth of mycorrhizal fungi *in vitro* [25].

The values of pH of MSM broth for the moulds over the period of biodegradation were slightly acidic, as values ranged from 4.40 to 5.30 for *Mucor* spp. and *A. flavus,* which ranged from 6.70 to 6.90, which is more acidic among the fungal isolates. *A. niger* and *F. oxysporum* steadily increase toward neutrality over the period of biodegradation. This result varies slightly for values reported by Lancaster *et al.* [26], who obtained pH values of 4.55 and 6.44 for *A. niger* and *Rhizoctonia* spp. during the biodegradation of herbicide-contaminated soils.

Increases in the biomass weight of the fungal isolates used for biodegradation indicate fungal growth in herbicide-containing medium. *A. niger* has the highest biomass growth (18%), followed by *F. oxysporum* (8.60%), *Mucor* spp. (8.90%), and *A. flavus (*9.90%) over a period of 12 days of biodegradation. Also, Ratcliff *et al.* [27] reported a transient increase in fungal propagules after glyphosate addition (50 mg/L). It is likely that the glyphosate provided nutrients for fungal growth, as evidenced by the significant increase in the microbial population. This conclusion is in agreement with Lancaster *et al.* [26], who found that after repeated application of glyphosate, microorganisms were better able to utilize it. The study by Partoazar *et al.* [28] concluded that glyphosate application may alter or increase soil microbial activity and population. Increased microbial activity may be beneficial or detrimental to plant growth, soil microbial ecology, and soil quality. Among the ten isolated species, four of the most tolerant fungi were used for biodegradation (*A. niger*, *A. flavus*, *Mucor* spp., and *F. oxysporum*), which were not or slightly influenced by herbicides up to 3%.

CONCLUSION

Findings made in this research revealed that most of the indigenous soil microflora has the potential to biodegrade glyphosate, whose product of biodegradation is neither harmful nor toxic. Biodegradation is a better and much more attractive method of mopping up herbicide-contaminated soil when compared to other conventional methods usually used; such as the use of incinerators and chemical methods, which are very costly and can degenerate into environmental problems.

In conclusion, *A. niger*, *A. flavus*, *Mucor* spp., and *F. oxysporum* can be employed as potentially effective fungal strains and environmentally safer alternative tools to protect the environment from the pollution of glyphosate-containing herbicides.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

All data and materials used for this study are available through the corresponding author on reasonable demand.

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AUTHOR'S CONTRIBUTION

O.A.A. conceived the idea for this research work and proposed the design. O.A.A., O.O.J., and J.R.J. conducted the experiments, analysis, and discussion of the results. O.A.A. drafted the original writing of this work, while O.O.J. and J.R.J. carried out a critical review of the revised manuscripts. A.M.A. and F.B.A. carried out proofreading and editing of the manuscript. All authors read and approved the final manuscript.

COMPETING INTEREST

The authors declare that there are no competing interests. All authors give their consent to the publication of this research work.

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