

BIOCONTROL POTENTIALS OF CRUDE EXTRACTS OF SOIL FUNGI ON  
*AMARANTHUS HYBRIDUS* AND *PHYLLANTHUS AMARUS*

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**Abstract:** In a pot experiment, two fungal strains from soil, *Aspergillus fumigatus* and *Penicillium citrinum*, were evaluated for their mycoherbicidal properties on *Amaranthus hybridus* and *Phyllanthus amarus* using the biomass reduction method. The experiment was set up in a completely randomised block design made up of two weed species exposed to 20 treatments which consisted of the concentrated crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* at three levels as well as positive and negative controls, each in three replications. The percentage biomass yields of *Amaranthus hybridus* and *Phyllanthus amarus* were determined at 1, 3, 5 and 7 days after application and biomass reductions were calculated. Data collected were subjected to analysis of variance and significant means were separated using Duncan's multiple range test. Infections of the weeds occurred within 48 hours after the application of the crude extract treatments. Significant differences ( $p \leq 0.001$ ) were observed in the percentages of biomass yields of the two weed species, especially at the highest inocula concentration (5% w/v) of the crude extracts. The percentages of biomass yields of *Amaranthus hybridus* were 88.58% and 88.91%, while 69.79% and 81.34% were recorded for *Phyllanthus amarus* after the application of the concentrated extracts of *Aspergillus fumigatus* and *Penicillium citrinum*, respectively. The study shows that the concentrated crude extracts of both *Aspergillus fumigatus* and *Penicillium citrinum* had the potentials for use as biocontrol agents, with the fact that extracts of *Penicillium citrinum* had the greatest impact on the biomass yields of the two test weeds.

**Key words:** soil fungi, mycoherbicidal properties, crude extracts, biocontrol and weeds.

### Introduction

The management of weeds requires the use of suitable techniques and approaches to reduce economic expenses and increase crop productivity. *Amaranthus hybridus* and *Phyllanthus amarus* are weed species belonging to

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*Amaranthaceae* and *Euphorbiaceae* family, respectively. The two weed species are annual herbs that reproduce from seeds and can grow up to 60cm to 80cm high. *Amaranthus hybridus* is highly hybridised and exhibits a wide range of colour variations. It is cultivated or harvested from the wild and is eaten as a vegetable in some parts of West Africa, while *Phyllanthus amarus* is a common weed of cultivated fields which is very widespread in West Africa. Several efforts have been made to reduce the menace of weed infestation by adopting different control measures, but chemical control has been internationally accepted to be most effective in reducing weed infestations. There are increasing constraints in the use of synthetic herbicides because weeds are becoming resistant, some products are being removed from sale as a result of re-evaluation, and rules governing usage are being tightened. There is also an increasing public demand for organic produce, free from synthetic residues. Bioherbicides serve a more important role as a complimentary component in successful integrated management strategies (Hoagland et al., 2007), and not as a replacement for synthetic herbicides and other weed management tactics (Singh et al., 2006). Although the research on mycoherbicides started in the 1940s in the developed countries where soil and rhizospheric fungi have been screened for bioactive compounds with specific interest in secondary metabolites produced biotechnologically for agrochemical industry, there has been a dearth of information on the bioherbicidal potentials of indigenous soil fungi in soils of the southern Guinea savanna (SGS) agro-ecological zone of Nigeria. This study was therefore conducted to evaluate the biocontrol potentials of *Aspergillus fumigatus* and *Penicillium citrinum* isolated from a SGS soil on *Amaranthus hybridus* and *Phyllanthus amarus*.

### **Materials and Methods**

#### Description of the experimental site and collection of soil samples

The experiment was conducted in the screen house of the Faculty of Agriculture, University of Ilorin, Kwara state, Nigeria. Soil samples were collected randomly from 0–15 cm depth in a farmland near the screen house cropped with Citrus and Moringa plants with the use of a soil auger into polyethylene bags. The samples were bulked, thoroughly mixed and air-dried.

#### Determination of some physicochemical properties of the soil

Moisture content was determined using the weight loss-on-ignition method; pH of the soil sample in water and pH in 1N KCl (1:2.5) were determined as described by Bates (1954); percent organic carbon and organic matter were determined using the Walkley-Black method as described by Jackson (1996); total nitrogen determination was made by the micro Kjeldahl distillation method as

described by AOAC (1999) and; determination of available phosphorus in soil was made by the Bray 1 method (Bray and Kurtz, 1954).

#### Isolation of fungi

The serial dilution agar plating method as described by Onyegeme-Okerenta et al. (2009) was adopted. Ten-fold serial dilutions of the soil suspension from each soil samples were made in sterile water. Potato dextrose agar (PDA) medium was used for the isolation of the fungi from the soil samples. Sterilized PDA plates were inoculated with  $10^{-3}$  dilutions of soil samples and incubated for 10 days at  $28^{\circ}\text{C} \pm 2$ . After ten days, the growth of the different fungal strains was observed. Each fungal isolate was then sub-cultured on fresh medium to obtain pure cultures.

#### Production of fungal inocula

Fungal inocula of *Aspergillus fumigatus* and *Penicillium citrinum* were produced on Czapek Dox broth. Mycelial discs of pure cultures were obtained using a 10mm sterile cork borer. The medium, prepared in 2-liter flasks, was then inoculated with twenty of the 10mm mycelial discs of pure fungal cultures and incubated in a rotary incubator at 150 rpm,  $28^{\circ}\text{C}$  for 7 days. Harvested mycelial batches from the flasks were then filtered using Whatman filter paper No. 40 and oven dried at  $80^{\circ}\text{C}$  for 24 hr. The dry weights were recorded in order to determine mycelial biomass (referred to as dry mycelium equivalents). The fermentation product was homogenized in 3–4 L aliquots with an electric blender prior to spray application on the weeds.

#### Pot experiment

The experimental design used was a completely randomized design (CRD) made up of twenty treatments which consisted of two weed species treated with the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* at three levels with positive and negative controls each in three replications.

The viable weed seeds were obtained from the wild, surface sterilized with 0.05% NaOCl for 5 minutes and rinsed with sterile distilled water. They were germinated ( $28^{\circ}\text{C}$ ) on moistened filter paper in Petri dishes and were later transferred into pots containing sterilized soils supplemented with the addition of poultry droppings. The different concentrations of the crude extracts were 1.0 $\times$ , 0.5 $\times$ , and 0.1 $\times$ , where 1.0 $\times$  concentration contained equivalent of 50g of mycelium (dry weight basis)  $\text{L}^{-1}$  and a positive control containing the surfactant only and a negative control containing only sterile water. The sprays were applied with the use of hand held sprays. After treatment, plants were monitored at daily intervals for disease development for 7 days. Plants were excised at the soil line, oven-dried for

24 hours at 85°C and the percentage of biomass reduction was determined at 0, 3, 5, and 7 days after application (DAA).

#### Data analysis

The reductions in biomass of the selected weeds in the pot experiment were calculated for each of the treatments. The data were subjected to analysis of variance at 5% probability level. The significant means were separated using Duncan's multiple range test.

### Results and Discussion

#### Physicochemical analyses of the soil

Table 1 shows some physicochemical properties and nutrient status of the soil used for the study. The characteristics of the soil agree with the assertions of Osundare (2009) that soils of the southern Guinea savanna are mostly classified as Alfisol with majority of them falling within the sandy-loam textural class. The fertility levels of these soils were reported to be usually low creating the need for constant augmentation through the addition of either organic or inorganic fertilizers. The pH of the soil used for this study was slightly acidic while the available phosphorus level was within the high range. The physicochemical properties of soil and its nutrient 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 (Rohilla and Salar, 2012).

Table 1. Some physicochemical properties of soil.

Source of soil sample	Organic matter (%)	Organic carbon (%)	pH		Nitrogen (%)	Available phosphorus (ppm)	Moisture content (%)
			Water	KCl			
*Unilorin T & R Farm	0.86	0.50	6.3	5.9	0.06	7.12	19.05

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Effects of the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* on *Amaranthus hybridus* and *Phyllanthus amarus*

Tables 2 and 3 show the main and the interactive effects of the crude extract formulations of *Aspergillus fumigatus* and *Penicillium citrinum* on the biomass yields of *Amaranthus hybridus* and *Phyllanthus amarus*, respectively. Significant differences ( $p \leq 0.001$ ) were observed in the reduction of the biomass yields of the weed species at 5 and 7 days after the application of the treatments as shown in Table 2.

Table 2. Main effects of the crude extract formulations of *Aspergillus fumigatus* and *Penicillium citrinum* on the biomass of *Amaranthus hybridus* and *Phyllanthus amarus* at various days after application.

Treatments	Biomass yield reduction (g) of the selected weeds at various days after application (DAA)			
	1	3	5	7
<b>Weeds</b>				
<i>Amaranthus hybridus</i>	0.92	0.49	0.26	0.41
<i>Phyllanthus amarus</i>	0.62	0.47	0.41	0.20
SED	0.18	0.11	0.04	0.04
LSD	0.36	0.21	0.08***	0.09***
<b>Levels</b>				
Crude extract formulation of <i>Aspergillus fumigatus</i> (% w/v)				
5	2.86	1.46	0.617	0.36
2.5	1.94	1.05	0.462	0.35
0.5	1.56	0.72	0.595	0.30
0 (+ ve)	0.38	0.39	0.68	1.31
0 (- ve)	0.40	0.42	0.52	1.19
Crude extract formulation of <i>Penicillium citrinum</i> (% w/v)				
5	0.28	0.15	0.09	0.06
2.5	0.30	0.20	0.21	0.07
0.5	0.07	0.11	0.13	0.25
0 (+ve)	0.16	0.20	0.19	0.25
0 (-ve)	2.86	1.46	0.62	0.36
SED	0.40	0.24	0.09	0.10
LSD	0.81***	0.48***	0.18***	0.20
<b>Interaction</b>				
Weeds × Levels				
SED	0.56	0.53	0.12	0.14
LSD	1.14	0.67	0.25**	0.29**

It was observed that the highest concentrations of the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* were more efficient than the lower concentrations. The highest concentrations of the crude extract (5% w/v) of *Aspergillus fumigatus* and *Penicillium citrinum* had 72.74% and 78.68% biomass yield reduction, respectively. It was observed that the crude extracts of *Penicillium citrinum* significantly ( $P \leq 0.001$ ) reduced the biomass of the weed species and it was more effective than the crude extracts of *Aspergillus fumigatus*. Gupta (1998) reported that the aggressiveness and percentage rot of *Penicillium citrinum* were found to be directly correlated with the amount of citrinin production. However, the efficacy of the highest concentrations began to reduce at 5 and 7 days after the application of the crude extracts as shown in Table 2. This might be attributed to the age of the plants as reported by Wolf (2011) and Boyette et al. (2014) that the efficacy of mycoherbicides decreases as the plants mature. Ghorbani et al. (2002)

reported that the efficacy of *Ascochyta caulina* was substantially reduced on older plants when applied on common lambsquarters (*Chenopodium album*).

Table 3. Interactive bioherbicidal effects of the different levels of the crude extract formulations of *Aspergillus fumigatus* and *Penicillium citrinum* on the biomass of four common weeds at various days after application.

Weeds	Treatments	Levels (% w/v)	Biomass yield reduction (g) of the selected weeds at various days after application (DAA)			
			1	3	5	7
<i>Amaranthus hybridus</i>	Crude extract ( <i>Aspergillus fumigatus</i> )	5	3.09	1.48	0.49	0.26
“	“	2.5	2.63	1.40	0.45	0.34
“	“	0.5	2.16	0.92	0.73	0.35
“	Crude extract ( <i>Penicillium citrinum</i> )	5	0.32	0.17	0.12	0.80
“	“	2.5	0.37	0.19	0.10	0.38
“	“	0.5	0.28	0.20	0.12	0.74
“	“	0 (+ve)	0.07	0.12	0.13	0.31
“	“	0 (-ve)	0.08	0.14	0.18	0.29
<i>Phyllanthus amarus</i>	Crude extract ( <i>Aspergillus fumigatus</i> )	5	2.63	1.43	0.75	0.46
“	“	2.5	1.26	0.70	0.47	0.35
“	“	0.5	0.97	0.53	0.46	0.25
“	“	0 (+ve)	0.06	0.46	0.58	0.15
“	“	0 (-ve)	0.23	0.33	0.40	0.13
“	Crude extract <i>Penicillium citrinum</i>	5	0.25	0.15	0.10	0.12
“	“	2.5	0.19	0.10	0.07	0.15
“	“	0.5	0.31	0.20	0.31	0.08
“	“	0 (+ve)	0.06	0.46	0.58	0.15
“	“	0 (-ve)	0.23	0.33	0.40	0.13
		SED	0.56	0.53	0.12	0.14
		LSD	1.14	0.67	0.25**	0.29**

The percentage reduction in biomass yields of the weed species at 5% w/v concentration was in the order: *Phyllanthus amarus* 60.40% at 3DAA > *Amaranthus hybridus* 52.29% at 7DAA > *Phyllanthus amarus* 47.90% at 5DAA. The higher percentage reduction in the biomass yield of *Phyllanthus amarus* might be attributed to the composition of the plant. The morphological classification is the most important and useful in weed control as morphological characters of a plant are closely related to herbicidal absorption, retention, and translocation. The weeds belonging to the same group are likely to have the same kind of response to specific herbicides or cultural or mechanical methods. Significant differences ( $p \leq$

0.001) were observed in the interactive effects of the different levels of application of crude extracts and the weed species (Table 3).

The two highest concentrations (5% w/v, 2.5 % w/v) of crude extracts were more efficient than the lowest concentration (0.5 % w/v). There were no significant differences between the positive and negative controls. The 5% w/v and 2.5% w/v concentrations of *Penicillium citrinum* crude extracts were more efficient than 0.5% positive and negative controls in reducing the biomass of both *Amaranthus hybridus* and *Phyllanthus amarus*.

At 7 days after application of the crude extracts, the highest percentage reductions in biomass yields (88.91% and 81.34%) were observed on *Phyllanthus amarus* while 88.58% and 69.79% were recorded for the reduction in biomass yield of *Amaranthus hybridus* by the highest crude extract formulation of *Penicillium citrinum* and *Aspergillus fumigatus* respectively. Graham et al. (2004), Peng and Wolf (2008) reported that disease response to an increased dose of bioherbicide agents may be non-linear and substantially higher inoculum doses can be required for noticeable efficacy improvement.

### Conclusion

This study has shown that crude extracts of both *Penicillium citrinum* and *Aspergillus fumigatus* have the potentials to be used in controlling weeds as shown by reduction in the biomass yields of both *Amaranthus hybridus* and *Phyllanthus amarus* with application of crude extracts of the soil fungi. The crude extract formulations of *Penicillium citrinum* are more effective on the weed species than the crude extract formulation of *Aspergillus fumigatus*. Further studies for determination of the effective biological metabolites responsible for the herbicidal properties of the fungal crude extracts are recommended.

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POTENCIJALI SIROVIH EKSTRAKATA ZEMLJIŠNIH GLJIVA U  
BIOKONTROLI KOROVA  
*AMARANTHUS HYBRIDUS* I *PHYLLANTHUS AMARUS*

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R e z i m e

Proučavana su mikoherbicidna svojstva dva soja gljiva iz zemljišta *Aspergillus fumigatus* i *Penicillium citrinum* na korovske vrste *Amaranthus hybridus* i *Phyllanthus amarus*, u ogledu u sudovima, korišćenjem metoda redukcije biomase. Ogled je postavljen po slučajnom blok sistemu sačinjenom od dve vrste korova sa 20 tretmana, koji su se sastojali od koncentrovanih sirovih ekstrakata dobijenih iz zemljišnih gljiva *Aspergillus fumigatus* i *Penicillium citrinum* na tri nivoa, kao i od pozitivne i negativne kontrole, svaki u tri ponavljanja. Prinosi biomase korova *Amaranthus hybridus* i *Phyllanthus amarus* izraženi u procentima smanjenja su određeni posle 1, 3, 5. i 7. dana od primene. Prikupljeni podaci su obrađeni analizom varijanse i značajne srednje vrednosti odvojene su korišćenjem Dankanovog testa višestrukog opsega. Infekcije korova su se javile u toku 48 sati nakon primene tretmana sa sirovim ekstraktima. Uočene su značajne razlike ( $p \leq 0,001$ ) u procentima smanjenja prinosa biomase dve korovske vrste, posebno pri najvećoj koncentraciji inokuluma (5% w/v) sirovih ekstrakata. Prinos biomase korova *Amaranthus hybridus* bio je 88,58% odnosno 88,91% manji u odnosu na kontrolu, dok je 69,79% odnosno 81,34% smanjenja zabeleženo za korov *Phyllanthus amarus* posle primene koncentrovanih ekstrakata dobijenih iz gljiva *Aspergillus fumigatus* i *Penicillium citrinum*. Istraživanje pokazuje da su koncentrovani sirovi ekstrakti dobijeni iz gljiva *Aspergillus fumigatus* i *Penicillium citrinum* imali potencijale da se upotrebe kao biokontrolni agensi, uz činjenicu da su ekstrakti dobijeni iz gljive *Penicillium citrinum* imali najveći uticaj na smanjenje prinosa biomase dve vrste ispitivanih korova.

**Ključne reči:** zemljišne gljive, mikoherbicidna svojstva, sirovi ekstrakti, biokontrola i korovi.

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