EFFECTS OF CRUDE EXTRACTS OF *ASPERGILLUS FUMIGATUS* AND *PENICILLIUM CITRINUM* ON THE BIOMASS OF *Eleusine Indica* (L.) Gaertn

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ABSTRACT

In a pot experiment, two fungal strains namely Aspergillus fumigatus and Penicillium citrinum were evaluated for their herbicidal properties on the biomass of Eleusine indica using the biomass reduction method. Crude extracts of Aspergillus fumigatus and Penicillium citrinum made up of 10 treatments at three levels each with positive and negative controls in three replications in a Completely Randomised Block Design was adopted. Infections were evident within 48 hours after the application of the various treatments. At 1, 3, 5 and 7 days after application of the treatments, the percentage biomass yields and biomass reductions of E. indica were determined. The data collected were subjected to analysis of variance and the means separated using Duncan Multiple Range Test. The percentage reductions in biomass yields (88.46% and 35.61%) of Eleusine indica especially at the highest inocula concentration (10^{10} spores/ml) of the crude extracts showed significant differences ($p \leq 0.001$). The results indicate that both crude extracts possess the potentials for herbicidal control of Eleusine indica with evidence that the concentrated crude extracts of Aspergilus fumigatus had the greatest impact on its biomass yields. Therefore, it is recommended that further work be carried out on the purification of both crude extracts to confirm the effective biological metabolites accountable for their herbicidal properties and also evaluate these herbicidal properties on other weed species adopting different time and methods of application.

Key words: Fungal strains, Herbicidal properties, Biological metabolites, Concentrated crude extracts, Biomass yields and Biomass reductions.

INTRODUCTION

Weeds are peculiar category of pests that greatly limit the production of major crops and also compete with them for nutrients, light and moisture resulting in significant loses in crop yield (Ofor *et al.,* 2009; Takim *et al.,* 2012). In West Africa sub-region, arable crop production is experiencing serious economic losses due to crop pests (Takim *et al.,* 2012). In minimizing loses due to pests and diseases, crop farmers have opted to use synthetic chemicals often-times associated with adverse effects on the environment and human health (Owonbo *et al.* 2014). These synthetic chemicals are toxic in nature affecting both the target and non-target species of plants and animals. Hence, the need to essentially subject them to safe and judicious use (Dahiru *et al.,* 2014). Oruonye & Okrikata (2010) confirmed that the application of synthetic agro-chemicals to control crop enemies has a lot of adverse ecological impact. In the application of herbicides, the optimal rate and time of application should be greatly considered owing to the great concern on environment and public health and the recent development of resistant weeds (Zhang *et al.,* 2013) while maintaining weed control at an acceptable level and minimizing injuries to crops (Nurse *et al.,* 2007). One of the most important tools to minimize the indiscriminate release of herbicide into the environment is to use herbicides at reduced rates which could also ultimately lower the cost of weed control (Swanton and Weise, 1991; Zhang *et al.,* 2013).

The increasing need to maintain a healthy and sustainable environment calls for non-chemical approaches to weed management. There is an increasing recognition that non-chemical methods of weed control have numerous advantages both for man and his environment despite the considerations by many countries of the world that herbicides are the main means of weed control (Bond *et al.,* 2003). In this regard, the deliberate use of natural enemies to suppress the growth or reduce the population of the weed species (biological approach) is gaining momentum (Boyette *et al.,* 2014).

Studies have been conducted by various scientists on the potentials of fungal isolates as biocontrol agents against several weed species in the developed countries. For instance, Elzein *et al.* 2006 & Elzein *et al.* 2010 studied seed coatings containing *Fusarium oxysporum* isolates to control *Striga*, and discovered that a 40% gum arabic seed coating combined with dried chlamydospores to be the most effective combination of seed coating and inoculum type for causing disease in *Striga*. Zhao & Shamoun (2006), also tested combinations of gelatin and potato dextrose broth concentrations for optimum efficacy of *Phoma exigua* to control salal (*Gaultheria shallon*), a perennial evergreen shrub. *Fusarium oxysporum* f. sp. *orthoceras* (FOO) known to suppress the root parasitic weed broomrape *(Orobanchecumana)* in sunflower. The use of microbially-derived compounds in biological control of weeds represent a promising alternative to the use of chemicals. Unfortunately, in Nigeria, little is known about the herbicidal properties of our indigenous soil fungi. This study was therefore

conducted to evaluate the biocontrol potentials of the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* on both the fresh and dry biomass of *Eleusine indica*.

MATERIALS AND METHODS

Experimental site description and collection of soil samples

Soil samples categorised as alfisols were collected from the top soil of four different farm sites within 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 (Fawole and Yahaya, 2017).

Isolation of fungal strains

Ten-fold serial dilutions of each soil sample were made in sterile distilled water. Potato Dextrose Agar (PDA) medium was used to isolate fungi from 1ml of 10^{-3} dilutions of soil samples. Inoculated plates were then incubated at 28^{o}C for ten (10) days (Onyegeme-Okerenta *et al.,* 2009; Fawole and Yahaya, 2017). After ten days, the growths of the different fungal strains were observed. The isolates were sub-cultured on fresh medium and further purifications were performed.

Production of fungal inocula

Czapex yeast broth (CYB) medium consisting of sucrose (30.0g), $NaNO_3$ (2.0g), Magnesium glycerophosphate (0.5g), KCl (0.5g), $FeSO_4$ (0.01g), K_2SO_4 (0.35g) were used for preparation of 1000 ml culture medium. The prepared medium was then autoclaved at 121ºC for 15 min and poured in separate conical flasks. Spore suspensions (0.1ml) obtained from 7 days old slant culture of *Aspergillus fumigatus* and *Penicillium citrinum* were inoculated in those separate flasks containing the media and the inoculated flasks were then transferred to shaking incubator for 20 days at 28^{o}C and 150 rpm as described by Namasivayam *et al.* (2014); Fawole and Yahaya (2017).

Extraction of fungal metabolites from broth medium

After the incubation period, 250µl of 40% HCl was added to each of the inoculated flasks to separate the media components. The fungal mycelia were ground using electric blender and equal volumes of ethyl acetate were added. The contents in the various flasks were mixed separately for 40 minutes and the mycelia filtered with Whatman filter paper No. 1. The filterates were then transferred into the separating funnel for separation of the organic layers containing the metabolites. These were washed with 2M brine solution to remove the

impurities and anhydrous Na_2SO_4 was used to dehydrate the organic layers which were again filtered and the recovered organic layers containing the crude metabolites were concentrated on a rotary evaporator at 45^{o}C. The concentrated extracts were dissolved in sterile di-methyl sulfoxide (DMSO) and used to assay for herbicidal activity as described by Namasivayam *et al.* (2014).

Experimental design

Two concentrated extracts of *Aspergillus fumigatus* and *Penicillium citrinum* made up of ten (10) treatments at three levels (10^8, 10^9, 10^{10} spores/ml) each with positive and negative controls in three replications in a Completely Randomised Design (CRD) was adopted. The total number of pots used for the experiment were 30. The viable *Eleusine indica* seeds were obtained from the wild and surface sterilized with 0.05% NaOCl for 5 minutes and rinsed with sterile distilled water. They were germinated at 28^{o}C on moistened filter paper in petri dishes and later transferred into pots containing sterilized soils which were supplemented with the addition of poultry droppings cured by dry ashing (Fawole and Yahaya, 2017). The different concentrations of crude extracts were 10^8, 10^9, 10^{10} spores/ml with positive and negative controls containing di-methyl sulfoxide only (surfactant) and sterile water only, respectively. The sprays were applied with the use of hand held sprayers. After treatment, *Eleusine indica* plants were observed at daily intervals for the development of disease for 7 days. Plants were removed at the soil line, oven-dried for 24 hours at 85^{o}C and the percentage biomass reduction were determined at 0, 3, 5, and 7 days after application (DAA) as reported by Fawole and Yahaya (2017).

Data Analysis

The reductions in both the fresh and dry biomass of *Eleusine indica* were calculated for each of the treatments. The data were subjected to analyses of variance at 5% probability level and the means were separated using Duncan Multiple Range Test. The percentage reduction in biomass yield was also calculated for the highest inocula load and comparisons were made with both the positive and negative controls.

RESULTS AND DISCUSSION

Aspergillus fumigatus and *Penicillium citrinum* significantly reduced the biomass yields of *Eleusine indica* (Tables I & II). It was observed that the highest concentrations (10^{10} and 10^9 spores/ml) of the crude extracts formulation of *Aspergillus fumigatus* and *Penicillium citrinum* were the more efficient than the lower concentrations (10^8 spores/ml) and the controls. However, the efficacy of the highest concentrations begins to reduce after 5DAA of the crude extracts. This might be attributed to the age of the plants as reported by Boyette *et al.* (2014) that the efficacy of mycoherbicides decreases as plants mature. Imoloame (2014) opined that

the use of herbicides integrated with supplementary hoe weeding will be more effective than the application of only herbicides in the control of weeds throughout the crop life because it will significantly better the growth and results in higher yield of the crop especially for farmers in the Southern Guinea Savanna of Nigeria. At 7DDA, the dry biomass of *E. indica* significantly reduced by 35.61% and 88.46% treated with crude extracts of *P. citrinum* and *A. fumigatus* as compared with 36.71% and 49.29% of the positive control and negative control respectively.

The efficacy of the crude extract of *Aspergillus fumgatus* might be due to it ability to produce toxic metabolites which have the tendency to bypass many restraints of applying spore or mycelia suspensions Amusa (2005). However, he further stated that the application should be made at the most susceptible stages of plant growth and also that the mass production of the microbial toxins via fermentation or otherwise will probably be much easier than that of spore production.

CONCLUSION AND RECOMMENDATION

This study revealed that both *Penicillium citrinum* and *Aspergillus fumigatus* have the potentials to produce secondary metabolites that are able to control narrow leaf weeds as shown by the reduction in the biomass yields of *Eleusine indica* with the application of crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum*. However, the crude extract formulations of *Aspergillus fumigatus* were more effective on the *Eleusine indica* than that of *Penicillium citrinum*. Therefore, further studies for the determination of the effective biological metabolites responsible for the herbicidal properties of the fungal crude extracts and the appropriate time and methods of application are recommended.

Table I: Main effects of the crude extract formulations of A*spergillus fumigatus* and *Penicillium citrinum* on fresh biomass of *Eleusine indica* at various days after application

Treatments	Fresh biomass (g) of *Eleusine indica* at the various days after application (DAA)			
	1	3	5	7
Crude extract formulation of *Aspergillus fumigatus* (spores/ml)				
10^{10}	2.33	1.16	1.23	0.78
10^{9}	2.78	1.91	1.50	1.16
10^{8}	2.90	1.97	2.16	1.39
0 (+ve)	3.30	2.01	1.57	2.56
0 (-ve)	4.09	2.18	2.25	2.80
Crude extract formulation of *Penicillium citrinum* (spores/ml)				
10^{10}	2.18	1.17	1.10	0.73
10^{9}	2.64	1.55	1.73	0.84
10^{8}	3.20	1.83	1.73	1.86
0 (+ve)	2.98	2.19	2.03	2.30
0 (-ve)	4.40	2.50	2.03	2.96
SED	0.91	0.49	0.33	0.48
$LSD_{0.05}$	ns	ns	0.69*	0.99***

Keys ns: Not significant ***: 0.1% significant level **: 1% significant level 0 (+ve): No culture, surfactant only 0 (-ve): No culture, no surfactant, sterile water only

Table II: Main effects of the crude extract formulations of A*spergillus fumigatus* and *Penicillium citrinum* on oven-dried biomass of *Eleusine indica* at various days after application

Treatments	Oven-dried biomass (g) of *Eleusine indica* at the various days after application (DAA)			
	1	3	5	7
Crude extract formulation of *Aspergillus fumigatus* (spores/ml)				
10^{10}	1.63	0.65	0.59	0.69
10^{9}	1.74	0.74	0.72	0.85
10^{8}	1.56	0.75	0.83	1.02
0 (+ve)	1.66	0.78	0.78	0.94
0 (-ve)	1.79	1.25	1.10	1.38
Crude extract formulation of *Penicillium citrinum* (spores/ml)				
10^{10}	1.06	0.10	0.05	0.26
10^{9}	1.19	0.13	0.12	0.27
10^{8}	1.42	0.04	0.54	0.75
0 (+ve)	1.77	1.47	1.29	1.34
0 (-ve)	2.93	2.44	2.03	2.25
SED	0.56	0.40	0.28	0.45
$LSD_{0.05}$	ns	0.85***	0.59***	0.94*

Keys ns: Not significant ***: 0.1% significant level 0 (+ve): No culture, surfactant only 0 (-ve): No culture, no surfactant, sterile water only

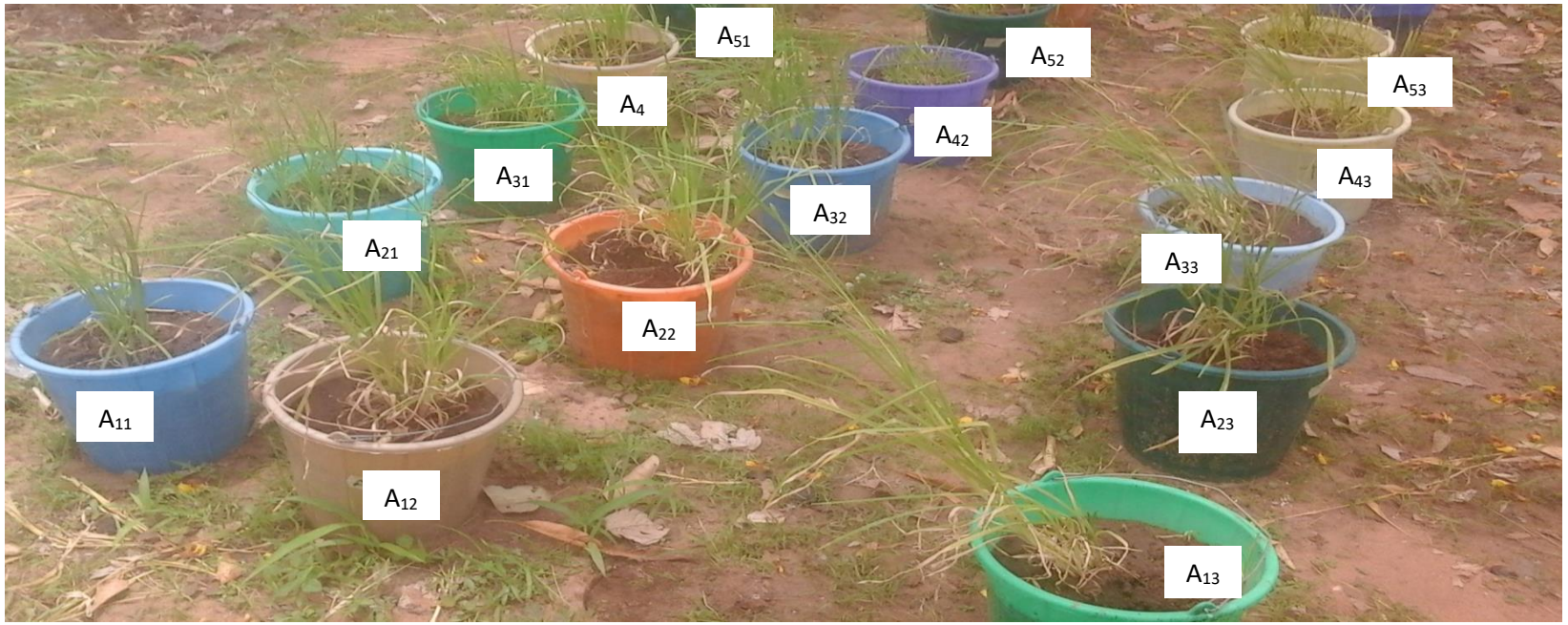

Figure I: Effect of the crude extract formulations of A*spergillus fumigatus* and *Penicillium citrinum* on the biomass *Eleusine indica*

Keys

A_{11}, A_{12}, A_{13} = Crude extract formulation of *Aspergillus fumigatus* at 10^{10} spores/ml

A_{21}, A_{22}, A_{23} = Crude extract formulation of *Aspergillus fumigatus* at 10^{9} spores/ml

A_{31}, A_{32}, A_{33} = Crude extract formulation of *Aspergillus fumigatus* at 10^{8} spores/ml

A_{41}, A_{42}, A_{43} = No culture, surfactant only (+ve)

A_{51}, A_{52}, A_{53} = No culture, No surfactant (-ve)

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