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Biodegradation potentials of soil-borne fungal cultures grown on haloxyfop R methyl ester herbicide medium

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Abstract

The heterotrophic and haloxyfop-R methyl ester utilizing fungal counts associated with two top soil samples was determined. Five (5) fungal isolates; Aspergillus niger, Candida sp., Trichoderma sp., Fusarium sp., and Alternaria sp. Were cultured from the soil samples using serial dilution as well as pour plate techniques. These isolates were sub-cultured and screened for their ability to use haloxyfop-R methyl ester as sole source of metabolic energy as well as using the turbidimeteric method. Two of the screened fungal isolates which exhibited maximal optical density (OD) difference values; A. niger and Fusarium sp. Were selected and utilized for the subsequent degradation/shake flask experiment. Growth profile parameters which included; pH, OD, dissolved CO_2 and weighed dry fungal biomass were determined during the growth profiling test at a 96 hour interval for 16 days using appropriate methods and equipment. Mean soil pH values were 5.08 ± 0.02 and 4.62 ± 0.02 for samples A and B. The mean total heterotrophic fungal count was 8.7×10^3 cfu/g ± 1.5 for A and 1.6×10^4 cfu/g ±1.0 for B. The mean pH value observed for A. niger in the course of the growth profile test ranged from 6.0 ± 8.7 to 7.3 ± 8.0 and 7.3 ± 5.8. The mean OD values observed for A. niger and Fusarium sp. In the course of the shake flask experiment varied from 0.66 ± 7.1 to 1.96 ± 4.5 and 1.62 ± 5.4 to1.99 ± 3.6 respectively. With reference to the detection of mean dissolved CO_2 values, utilized as an indirect indicator of herbicide mineralization, the axenic cultured A. niger was the most effective amongst the growth profile fungal cultures.

Keywords: Axenic, Fungal consortium, Haloxyfop-R methyl ester, Mineral salt medium, Shake flask.

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INTRODUCTION

Pesticides are a diverse group of chemicals designed to control unwanted organisms, including weeds, insects, and rodents (Kavita and Geeta, 2014).These chemicals come in various forms, each targeting specific pests based on how they work (Kavita and Geeta, 2014).Pesticides offer several benefits including; increased crop yields and food production, significantly reduce diseases spread by insects, and protect crops from damage and infestation. This ultimately leads to higher quality food products (Agrawal *et al*., 2010).

A consequence of agricultural urbanization and mechanization has been an increase in the release of pollutants especially pesticides in our ecosystem to an estimated one billion pounds of chemicals on a global scale (Ortiz-Hernandez *et al*., 2013). In the past, landfills were the go-to method for cleaning up contaminated areas. However, this approach has proven to be inadequate. A more promising strategy involves either completely removing the pollutants or transforming them into harmless substances (Abo-Alkasem *et al*., 2023). Although effective, it has several drawbacks principally due to technological complexity, the cost implications on such a small-scale application (Vidali, 2001). Hence, an ecofriendly and efficient alternative method for remediation of pollutants and toxicants from the contaminated site is needed.

Biodegradation, a process whereby waste materials are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities is an option (Azubuike *et al*., 2016). Biodegradation essentially uses natural biological activities to destroy or neutralize contaminants. Based on the potential advantages of this process over traditional cleanup methods, research in this area is rapidly expanding (Bala *et al*., 2022). The core principle of biodegradation involves utilizing living organisms, like bacteria, fungi, or even plants, to turn environmental pollutants into less toxic forms. These naturally occurring decomposers can effectively break down or detoxify substances that pose a threat to human health and the environment (Vidali, 2001).

These microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. To achieve effective biodegradation, microorganisms must enzymatically attack the pollutants and convert them to harmless products (Ayilara and Babalola, 2023). Microorganisms are known to play a significant role in the transformation and degradation of pesticides. Even the most persistent pesticides can be partially metabolized to some extent by microbial cultures, either by utilization of the compounds as sources of energy or nutrients, or by co-metabolism with other substrates supporting microbial growth (Castillo *et al*., 2006).

Haloxyfop-R methyl ester, is a member of the aryloxyphenoxy-propionate herbicide (AOPPs) family based on 4-oxyphenoxypropanoic acid which serve as its primary skeletal form . Globally, they have been described as a widely utilized pesticide (Zhu *et al*., 2015). The compound is known to have a chemical formula of $C_{16}H_{13}CIF_3NO_4$ with a molecular weight of 375.7 g/mol is used in controlling the growth of weeds within areas of economic crops such as potatoes, leaf vegetables, onions, soya beans and other broad leaf plants are cultivated (Chen, 1999). The herbicide is usually absorbed by plant and hydrolyzed to haloxyfop which is translocated to the meristematic tissues, hindering the proliferation of the weed plant (Chen, 1999). Zhu *et al.* (2015) reported that AOPPs do not usually negatively affect economic crops but its residues can have deletrious impacts on non-target biota.

The utilization of microorganisms for pesticide degradation especially AOPPs removal from contaminated soils has shown to be environmental friendly and cost effective (Hussain *et al*. 2015). However, few studies have detailed the potential application of edaphic fungi in reducing and removing pesticides from soil habitats. This study was aimed evaluating the biodegradative potentials of phenotypically characterized soil edaphic fungal cultures cultured on Haloxyfop-R methyl ester liquid medium. In achieving this research objective, several axenic fungal isolates were screened for their herbicide degradative abilities and cultures which showed appreciable degradative potentials were subjected to shake flask tests utilizing the herbicide as sole carbon and energy source respectively. Results from this study would provide relevant information on the suspected abilities of the edaphic fungi to degrade Haloxyfop-R methyl ester at varying rates.

MATERIALS AND METHODS

Study area

Hundred grams (100g) of top soil samples (0-15cm) were collected from two (2) locations within the botanical garden of Department of Plant Biology and Biotechnology, University of Benin, Ugbowo Campus, Benin City using soil auger. The coordinates of the sampled sites were; A (N 06^0 23. 843′ E 005^0 36.978′) and B (N 06^0 23. 852′ E 005^0 36.967). The soil samples were placed into labelled polyethylene bags. The herbicide, Haloxyfop R Methyl ester in its granular form was purchased under the trade name of Gallant Super and was appropriately utilized for the biodegradative experiments.

Laboratory procedures

Ten (10) grams of the fresh soil sample was weighed into a 100 ml glass beaker. Twenty (20) milliters of a 0.01 M $CaCl_2$ solution was added and the suspension was stirred continuously for 20 minutes (Kalra and Maynard, 1991). The hydrogen ion concentration (pH) of each sample were measured using HACH digital pH meter.

The media used in the preliminary isolation of both heterotrophic and herbicide degrading fungi were; Potato dextrose agar (PDA) and modified Mineral Salt Medium (MSM). Serial dilution and pour plate method as described by Cappuccino and Welsh (2020) was used for isolating fungal isolates from soil samples using PDA. Soil homogenates were made by suspending 1g of soil each sample in 10ml of sterile water. Serial soil dilutions were done up to 10^7 with the aim of isolating fungal colonies discretely spaced from one and another on the respective agar plate. One (1) ml of the diluted aliquot was dispensed onto labeled sterile Petri dish using pour plate method conducted in an aseptic setting.

One (1) ml of an antibacterial agent solution; Erythromycin; 500mg in 10ml distilled water was dispensed into each Petri dish prior to pouring of 10 -15 ml of col molten agar as previously described by Obayagbona and Enabulele (2013). Morphological and macroscopic examination was used to identify fungal isolates. The colony length, the presence or absence of aerial mycelium, the colour and any other pigment as well as macro- morphological characteristics were evaluated (Diba *et al*., 2007).

For the culturing of Haloxyfop-R methyl ester degrading fungi, modified mineral salt medium (MMSM) as described by Okpokwasili and Okorie (1988), modified by the addition of 1% Haloxyfop-R methyl ester which served as carbon source was utilized . Zero point one (0. 1) ml of 10mg/L chloramphenicol was added to the plates to inhibit fungal proliferation (Rice and Hemmingsen, 1997).

Purification and identification of isolates
The fungal isolates were purified sub-culturing the various cultures onto freshly prepared PDA plates). Phenotypic identification of fungal cultures was conducted using morphological and macroscopic examination (Diba *et al*., 2007).

Preparation of standard cultures for axenic fungal isolates
Standard fungal broth cultures were prepared for the isolates adapting the method of Obayagbona and Enabulele (2013). One hundred (100) ml of mineral salt broth was dispensed into conical flasks and inoculated with each isolate from pure culture stock with the aid of a sterilized inoculating loop and incubated at 28°C for 24 hr. After incubation, the cultures were serially diluted up to 10^{-2} and 0.1ml of each was added into sterile Petri dishes and cool molten PDA was added to the inoculated plates and incubated at 37°C for 24 hr. Resultant plate counts were recorded and the values obtained were expressed as standard number of cells present in 1ml of the broth. This was utilized as the standardized culture.

Screening the ability of the bacterial isolates to utilize Haloxyfop-R methyl ester as sole carbon and energy sources
The method as described by Okpokwasili and Okorie (1988) was adapted to screen the ability of purified fungal isolates to utilize Haloxyfop-R methyl ester as sole carbon and energy source using the modified mineral salts medium (Mills *et al*., 1978). Nine (9) ml of prepared mineral salt medium (MSM) was dispensed onto a set of test tubes. One (1) gram of granular Haloxyfop-R methyl ester was added to each of the tubes and capped before autoclaving at 121^0C for 15 minutes. Upon cooling, each of the first set of tubes was inoculated with 0.1ml of standardized cell suspension of each of the bacterial isolates. One control tube remained un-inoculated. The inoculated tubes and control tube were incubated at 35°C for 10 days.

The optical density of the respective tubes was taken at day 0 and day 10 respectively using a spectrophotometer; Biobase model BK-UV1800PC (Biobase bioindustry, Shandong). The difference between each OD reading was documented and cultured tube which had the highest difference were adjudged to have the best screening potentials with respect to Haloxyfop-R methyl ester degradation.

Growth profile of pure and mixed consortium of fungal isolates in haloxyfop-R methyl ester medium
The growth profile of the fungal isolates which were selected from the screening test were determined by the adaption of a procedure earlier described by Okpokwasili and Okorie (1988). Two (2) litres of mineral salt medium was prepared (pH 7.2). A specific quantity; 247.5 ml of the medium was dispensed onto several 250 ml conical flasks and a measured amount of granular herbicide (2.5g) was added to each of the flasks.

The flasks were autoclaved at 121 ^{0}C for 15 minutes and upon cooling, 2 ml of a 24 hr mineral salt medium broth culture of the respective bacterial isolates were pipetted into each respective flask except the control flask, under aseptic conditions (Okpokwasili and Okorie, 1988). The flasks were incubated at 37°C for 13 days on an incubator shaker (Heidolph unimax 2010, Heidolph Company, Wood Dale) operated at 120 rpm. Each flask was analyzed for Haloxyfop-R methyl ester utilization and mineralization at a 96 hr interval identical to an incubation interval previously utilized by Obayagbona and Enabulele, (2013). The indicators of Haloxyfop-R methyl ester utilization and mineralization determined at a 96 hr interval included; Total dry filtered fungal biomass, pH, optical density (OD) and

dissolved CO_2. Duplicate samples from the culture and control flasks were analysed and a mean value was derived from the duplicate values.

pH determination
The pH of each culture flask was determined with the aid of a pH (Suntex SP-2100) meter. The electrode was first calibrated with freshly prepared pH buffers 4.0, 7.0 and 14.0. The calibrated electrode was dipped into each beaker containing the samples and steady readings were recorded.

Determination of optical density
This was also determined at 96 hour interval for 16 days. The parameter was analyzed with the aid of a spectrophotometer (BK-UV1800PC). Ten (10) ml of the sample was dispensed into a clean cuvette under aseptic conditions and steady OD values were recorded at specified wavelength.

Determination of dissolved CO_2 content
Dissolved CO_2 was determined 96 hour interval for 16 days using the titrimetric method as described by Bastola (2013).

Total dry filtered fungal biomass
The dry weight of the fungal biomass of the respective flasks at day 16 was ascertained *via* filtration and weighing of the fungal mycelial biomass using sterile and clean Whatman filter paper size 16, funnel as well as a sensitive weigh balance as previously described by Obayagbona and Enabulele, (2013). Prior to final weighing of the dried fungal biomass, the filter paper was pre-weighed and the weight was recorded. The respective weighed fungal biomass was oven dried at 80 ^{o}C for 24 hours prior to weighing of the dried biomass.

Statistical evaluation of the growth profile data
All the mean data obtained for the axenic fungal isolates and fungal consortium were subjected to one-way analysis of variance using the statistical software; SPSS version 22. Mean separation was also conducted using a post hoc test; Duncan's multiple range test at 0.05 level of confidence.

RESULTS AND DISCUSSION

Edaphic pH, fungal counts and screening result
The mean pH values of the soil samples were 5.08 ± 0.02 and 4.62 ± 0.02 for samples A and B. (Table 1). All the analyzed soil samples were acidic and this trend was similar to an observation reported by Obayagbona *et al.* (2023) with respect to soil samples collected from the same sampled area. The observed acidity of the top soil samples examined in this study was also in agreement with findings indicated by Obayagbona and Enabulele (2013) with regard to top soils obtained from the vicinities of several auto-mechanic workshops located in Benin city. Soil pH has been described as an important influencer of edaphic microbial communities as well as abiotic factors which include; nutrient and carbon availability (Rousk *et al*. 2009). The mean heterotrophic fungal counts and haloxyfop-R methyl ester utilizing fungal counts for sample A were 8.7 ×10^3 cfu/g ± 1.5 for sample A and 6.7 × 10^3 cfu/g ± 2.5 . The detection of these fungal counts for the respective samples were indicative of the ability of the soil samples to serve as a substrate supporting the growth and proliferation of different fungal species. Although, information pertaining to likely historical usage of Haloxyfop-R methyl ester or other members of the aryloxyphenoxy-propionate herbicide (AOPPs) group on the sites where the samples were collected was not obtained, the preliminary isolation of fungal colonies capable of herbicide utilization could infer the

widespread distribution of these herbicide utilizers within the edaphic sites. The range of the mean heterotrophic counts observed for the soil samples contrasted with fungal counts reported by Obayagbona and Enabulele (2013) with respect to top soil samples obtained from various municipal auto –mechanic yards.

Table 1: The pH and fungal counts of the soil samples collected from botanical garden

Soil samples	pH	Total heterotrophic fungal Count (cfu/g)	Total Haloxyfop-R methyl ester utilizing fungal count (cfu/g)
A	5.08 ± 0.02	$8.7 \times 10^3 \pm 1.5$	$6.7 \times 10^3 \pm 2.5$
B	4.62 ± 0.02	$1.6 \times 10^4 \pm 1.0$	$8.5 \times 10^3 \pm 1.5$

A total of 5 fungal species were isolated and tentatively identified from the analyzed soil samples. The isolates were *Aspergillus niger*, *Candida* sp., *Trichoderma* sp., *Fusarium* sp., and *Alternaria* sp. The isolation of *A. niger* and *Candida* sp., was in agreement with an earlier report by Obayagbona and Enabulele (2013) which revealed the isolation of these isolates from various top soil samples collected in Benin city. The isolation of these tentatively identified fungal cultures with the exception of *Fusarium* sp. contrasted with a report by Osazee *et al.* (2013) which revealed the isolation of fungal cultures such as *Mucor* sp. and *Saccharomyces* sp. from several top soils collected from different sites in Benin city. Based on the difference in optical density (OD) after seven days, two axenic fungal cultures; *A. niger* and *Fusarium* sp. were selected. These organisms exhibited the highest OD difference during screening and was utilized for the subsequent degradation/shake flask experiment. The ability of these isolates to cause the maximal OD difference could be attributed to their respective ability to utilize inherent metabolic machinery to assimilate the dissolved herbicide which served as both a source of carbon and energy. The non-selected fungal isolates (*Alternaria* sp. and *Trichoderma* sp.) exhibited a comparative lower OD difference and this trend could be attributed to the poor utilization of the dissolved herbicide as a carbon and energy source.

Table 2: Haloxyfop-R methyl ester utilizing capabilities of the fungal isolates

Fungal isolate	Difference in absorbance reading	Decision
Aspergillus niger	**1.374**	**Selected**
Alternaria sp.	0.184	Not selected
***Fusarium* sp.**	**0.586**	**Selected**
Trichoderma sp.	0.242	Not selected

Shake flask experiment

The mean pH value documented for *A. niger* in the course of the growth profile test ranged from 6.0 ± 8.7 at Day 12 to 7.3 ± 8.0 and 7.3 ± 5.8 at Day 0 and 4 respectively (Table 3). Mean pH values recorded for *Fusarium* sp. varied from 5.8 ±3.0 at Day 12 to 7.3 ± 8.2 at Day 4 (Table 3). The mean pH data recorded for the fungal consortium and control ranged from 6.1 ± 0.58 at Day 12 and 6.1 ± 4.7 at Day 16 to 7.3 ±1.7 at Day 4 as well as 7.2 ± 2.2 at Day 4, 7.2 ± 5.7 at Day 8, 7.2 ±1.7 at Day 12 and 7.2 ±1.3 at Day 16 to 7.3 ±1.3 at Day 0 (Table 3). The differences between the mean pH readings was not statistically significant ($p>0.05$).

During the shake flask experiment, it was observed that the mean pH values of the agitated seeded flasks decreased from neutral concentrations at Day 0 to acidic readings but the magnitude of the pH reduction was higher in the flask inoculated with *Fusarium* sp. as the lowest mean pH values were observed for these culture flasks (Table 3). This trend could be attributed to the increased accumulation of unidentified metabolites such as; dissolved gases

and organic acids culminating in increased acidity of the surrounding medium. The higher acidity of the examined culture medium harboring a proliferating axenic fungal isolate; *Fusarium* sp. in comparison with the flasks seeded with a consortium of *A. niger* and *Fusarium* sp. contrasted with an observation reported by Obayagbona and Enabulele (2013) which indicated higher pH values for consortia of filamentous fungi and yeasts in comparison to axenic fungal isolates cultured on hydrocarbon sludge-glucose media.

Table 3: Mean pH of the axenic and consortial fungal cultures obtained during the study

Axenic and consortial cultures	DAY 0	DAY 4	DAY 8	DAY 12	DAY 16
	Mean±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
A. niger	7.3 ± 8.0	7.3 ± 5.8	6.1 ± 8.1	6.0 ±8.7	6.2 ± 6.5
Fusarium sp.	7.2 ±1.4	7.3 ± 8.2	5.9 ± 0.36	5.8 ±3.0	6.0 ± 0.19
Fungal consortium*	7.2 ± 2.2	7.3 ±1.7	6.2 ± 6.4	6.1 ± 0.58	6.1 ± 4.7
Control	7.3 ±1.3	7.2 ± 2.2	7.2 ± 5.7	7.2 ±1.7	7.2 ±1.3

*mixture of *A. niger* and *Fusarium* sp.

The mean OD values observed for *A. niger* and *Fusarium* sp. in the course of the shake flask experiment varied from 0.66 ± 7.1 at Day 4 to 1.96 ± 4.5 at Day 16 and 1.62 ± 5.4 at Day 0 to1.99 ± 3.6 at Day 16 (Fig. 1). The range of mean OD values documented for the fungal consortium and control varied from 1.58 ± 4.7 at Day 0 to1.90 ±1.8 at Day 16 and1.50 ±1.9 at Day 16 to 1.53 ±1.6 at Day 4 (Fig. 1). The mean OD data recorded for the bacterial consortium during the growth profile study ranged from1.44 ± 0.01 at Day 7 to1.91 ± 0.003 at Day 3 (Fig. 3). The observed variations between the mean OD was statistically insignificant ($p>0.05$). The mean OD values can be regarded as an indirect measurement of fungal activity and growth in each of the seeded flasks and as such, a direct correspondence was observed between the weighed dry biomass content of the culture flasks and the OD values of the corresponding flasks. The axenic *Fusarium* sp. grown on the herbicide medium at Day 16 had maximal mean OD readings and the weighed dry biomass filtered from the same culture flask also had corresponding maximal dry weight amongst all the incubated flasks.

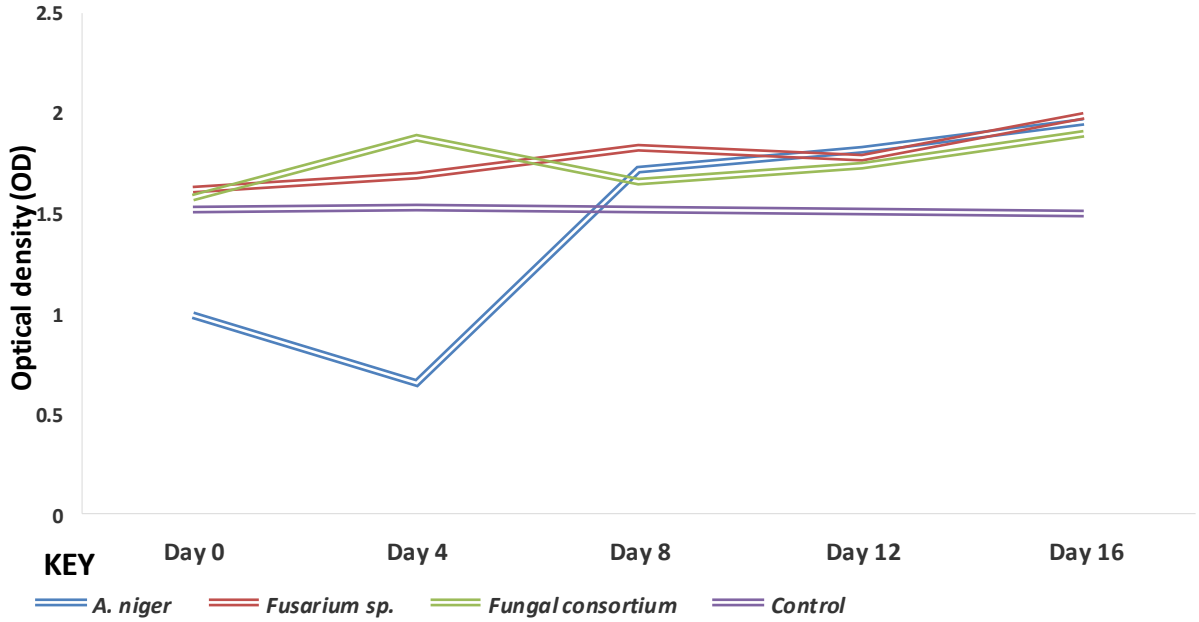

Fig. 1: Mean optical density values of the axenic and consortial fungal cultures

For the duplicate flasks seeded with axenic culture of *A. niger* and *Fusarium* sp. respectively, mean dissolved CO_2 data observed ranged from 2.2 mg/l ± 0.22 at Day 16 to 5.0 mg/l ± 0.16 at Day 4 (Fig. 2). The mean dissolved CO_2 values observed for the fungal consortium and the control flask varied from 2.9 mg/l ± 8.20 at Day 16 to 7.8 mg/l ± 0.17 at Day 4 and 8.4 mg/l ± 0.13 at Day 12 to 8.6 mg/l ± 0.13 at Day 4 (Fig. 2). The difference between the mean CO_2 values was statistically significant ($p<0.05$).

The dissolved CO_2 value of all the incubated flasks was utilized as a direct indicator of the mineralization rate(s) or complete biodegradation of the herbicide component of the growth medium by sole fungal or axenic or consortium of the two fungal species. The steady reduction in the dissolved CO_2 profile could have been suggestive of elevated herbicide degradation or mineralization rate by the proliferating fungal inoculant. As the control flasks were devoid of any fungal inoculant, minimal variations in the dissolved CO_2 content of the flasks were observed. This trend was suggestive of the active role, the fungal inoculants played in causing progressive fluctuations in the CO_2 content of the flasks in the course of these cultures proliferating and growing whilst utilizing the herbicide as carbon and energy source respectively. This trend was also a likely indication of the absence of abiotic herbicide degradation in the control flasks. The non-progressive reduction in the CO_2 content of the control flasks incubated for 16 days in this study was similar to an observation reported by Obayagbona *et al*. (2023) which indicated an identical trend for control flasks which also contained haloxyfop-R methyl ester but were incubated for 14 days.

Although the axenic *Fusarium* sp. had both the highest OD reading (Fig.1), dry weighed biomass amongst the axenic cultures (Fig. 3) as well as the lowest pH value (Table 3), the final CO_2 reading observed for this axenic culture was lower than the corresponding final value recorded for *A. niger* at Day 16. of the incubation period. This trend would suggest that *A. niger* might have been capable of conducting complete degradation or mineralization of more dissolved haloxyfop-R methyl ester moieties in comparison to *Fusarium* sp. or the fungal consortium during the shake flask experiment. However, this trend would suggest that although *A. niger* might have mineralized a higher amount of dissolved herbicide in the course of utilizing it as an energy or carbon source, *Fusarium* sp. was slightly better in assimilating the metabolic products arising from the complete degradation of the herbicide into its biomass culminating in the slightly higher weighed dried biomass observed for this culture (Fig. 3).

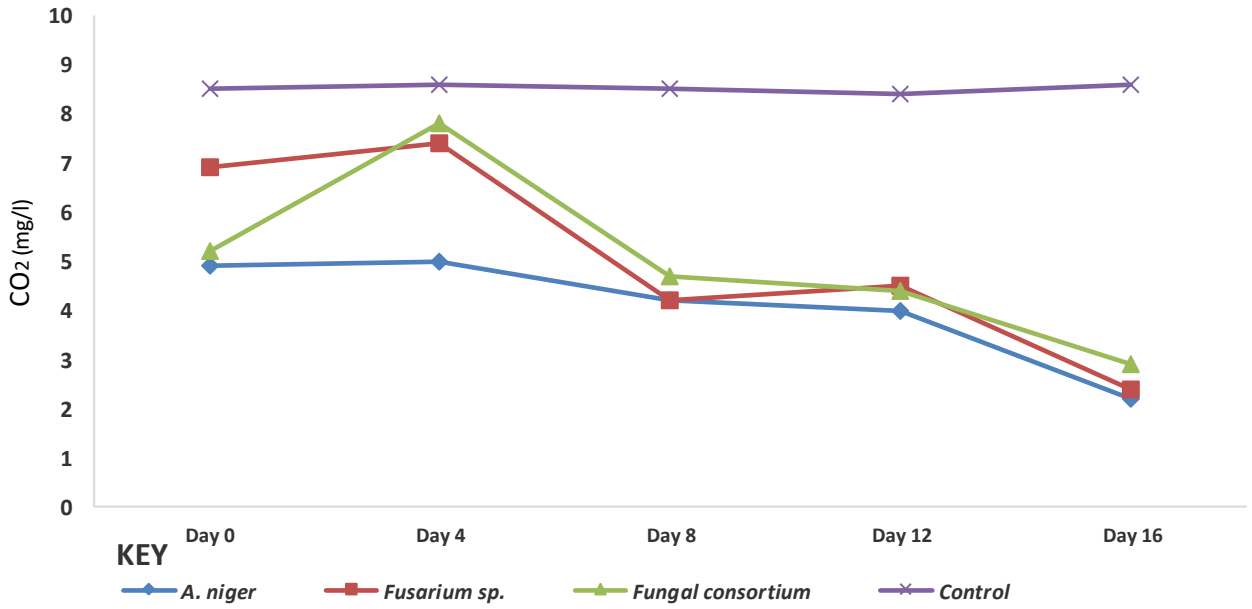

Fig. 2: Mean CO_2 optical density values of the axenic and consortial fungal cultures

Maximal dry weight (2.255g) value was observed for the fungal consortium while the axenic *A. niger* isolate had the lowest dry weight (1.85 g) at day 16 of the shake flask growth profile study (Fig. 3). Expectedly, the fungal consortium had the highest dry mycelial weight readings and this observation was in tandem with a reported trend by Obayagbona and Enabulele (2013) which revealed a higher dry weighed biomass for filamentous fungal consortium cultured on hydrocarbon sludge-glucose medium.

Although, the fungal consortium had the highest dry weight biomass reading, mean dissolved CO_z observed on Day 16 was comparatively lower than values recorded for both axenic fungal cultures. This trend could indicate that as a consortium, the fungal isolates mineralized lower amounts of the dissolved herbicide but when present as the respective sole cultures, either *A. niger* or *Fusarium* sp. completely degraded higher amounts of the dissolved herbicide. Another likely reason for the lower CO_2 of the flasks containing the fungal consortium might have been the production of undetected metabolites by any of the consortial members which might have hindered the mineralization of herbicide moieties by affected consortial member.

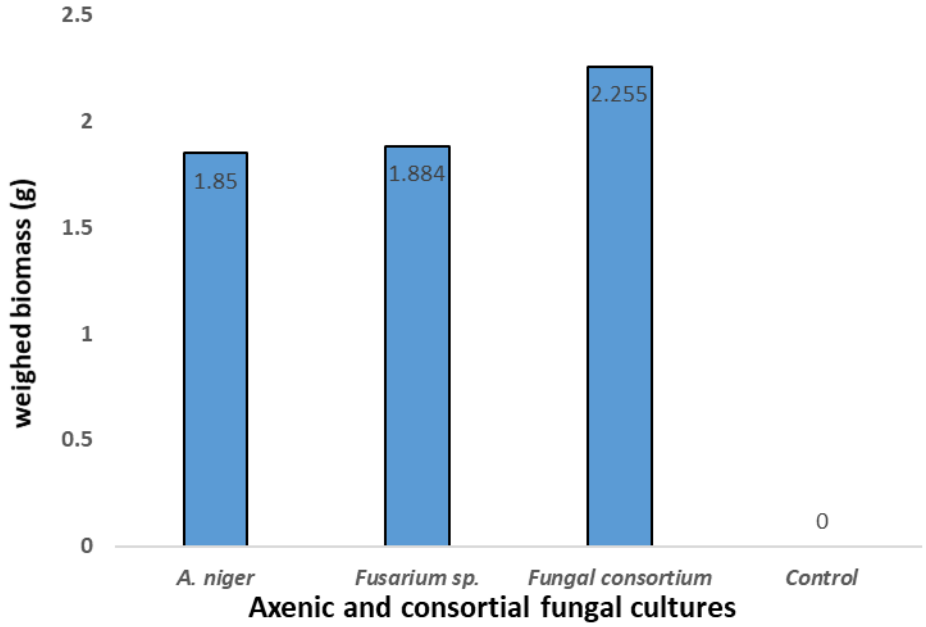

Fig. 3: Dry weight (g) of both the axenic and consortial fungal cultures on Haloxyfop-R methyl ester medium

CONCLUSION

A range of heterotrophic and haloxyfop-R methyl ester utilizing fungal counts were cultured from two acidic top soil samples. Two tentatively screened fungal isolates; *A. niger* and *Fusarium* sp. displayed varying degradative potentials when grown on Haloxyfop-R methyl ester modified medium. With reference to the detection of mean dissolved CO_2 values which was employed as an indirect indicator of herbicide mineralization, the axenic cultured *A. niger* was the most effective amongst the growth profile mycological cultures. However, in terms of final dry fungal biomass weight, which was used as a measure of the direct effect of culturing the respective fungal cultures on the herbicide medium, the fungal consortium had the maximal weighed dry biomass. A follow up study investigating the likely effect(s) of adding an extra carbon source such as starch or glucose on fungal mineralization or complete biodegradation of Haloxyfop-R methyl ester is recommended.

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