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Bioremediation of Spent Engine Oil Contaminated Soil by Using Fungus, *Penicillium sp.*

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Abstract. This study investigated the ability of *Penicillium sp.* to bio-remediate spent engine oil contaminated soil both *in vitro* and *in vivo*. In the *in vitro* assay, mycelium of a seven day old culture of *Penicillium sp*. grown on Sabouraud Dextrose Agar (SDA) was punched out using a 0.5mm Cork borer and inoculated on the centre of Petri dishes containing the spent and unspent engine oil and incubated for seven days and daily reading of the mycelia growth obtained using a metre rule. For the *in vivo* assay, soil received 0 (control), 20/180, 40/360, 60/540, 80/720 and 100ml/900mm concentrations/treatments (inoculation with mycelium of *Penicillium sp*.). Seeds of *Telfeira occidentalis* was sown on the soil and assessed for growth performance (plant height, leaf area (using a metre rule) and leaf count (number of leaves) for 7, 14, 21 and 28 Days after Planting (DAP). Results of the *in vitro* assay showed a significant increase ($p<0.05$) in the growth diameter of *Penicillium sp.* relative to control. Results of the *in vivo* assay showed that spent engine oil had no significant effect ($p<0.05$) on the growth performance of *T. occidentalis* at 7, 14, 21 and 28 DAP and on fresh and dry weight (g) 28 DAP relative to control. After 28 days of plant growth, the added spent engine oil was no longer detected. The plant began producing pods 61 DAP. This study showed that *Penicillium sp*. can biodegrade hydrocarbons present in spent engine oil and as such is a good tool for bioremediation.

Introduction

Bioremediation is a process that uses microorganisms such as fungi (mycoremediation) and green plants to remove contaminants such as oil from the environment, which could be *in-situ* or *ex-situ* [1]. Mycoremediation refers to the use of fungi to clean contaminated soil [2]. According to [3], to achieve a successful mycoremediation process, fungi must grow and survive in soils contaminated with oil. In oil contaminated sites, mycoremediation can be applied as a final clean up measure to further breakdown residual hydrocarbons as well as to improve soil quality [4]. Mycoremediation is a viable method that can be used to bio-remediate areas contaminated with pollutants because it is affordable and environmentally friendly [5].

According to Achuba et al. [6] and Wang et al. [7] spent engine oil is a brown-to-black liquid and a mixture of various chemicals such as aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products and heavy metal contaminants that come from engine parts as they wear out.

Similarly, Anoliefo et al. [8] reported that there are large amounts of hydrocarbons present in spent engine oil such as the highly toxic polycyclic aromatic hydrocarbon (PAH). Furthermore, Wei et al. [9] reported that spent engine oil in soil creates conditions that are unhealthy for plant growth such as heavy metal toxicity to poor aeration of soil. Odjegba and Sadiq [10] reported that contamination from spent engine oil is a major environmental challenge and is more widespread than crude oil pollution. According to Meinz [11] spent engine oil as a petroleum product contains potentially hazardous chemicals, especially the polycyclic aromatic hydrocarbons (PAHs), heavy metals and chemicals additives such as amines, phenol and benzenes while Ikhajiagbe and Anoliefo [12] reported that spent engine oil pollution can affect a vast area when they are carried by run-off during rainfall to nearby farms and Fetzer [13] reported that chemicals found in oil contaminated

soil can cause a reduction in the level of available plant nutrients and a rise to a toxic level of elements such as manganese.

Over the years, automobiles repairs and maintenance activities have been carried out by auto mechanics at the Uyo Mechanic Village located at Afa Ofot in Uyo Metropolis of Akwa Ibom State, Nigeria. The site is well known as a farming area where crops consumed around Uyo Metropolis, Ediene-Abak, Abak and Ikot Ekpene are harvested. In recent times, complaints by farmers have been received concerning loss in produce due to land pollution as a result of spent engine oil released by the auto-mechanics. In view of this and based on the menace caused by oil pollution on plants, a research on how to reclaim the farmland *ex-situ* was carried out using mycelium of *Penicillium sp*, a fungus also found within these areas. The objective of the present study therefore, was to test *Penicillium sp*. for its ability to bio-remediate spent engine oil contaminated soils both *in vitro* and *in vivo*.

Materials and Methods

Sources of Materials

Matured dry fluted pumpkin (*Telfeira occidentalis*) seeds were obtained in Uyo main market in Uyo Metropolis, while spent engine oil was obtained from the Uyo Mechanic Village, Afa Ofot in Uyo Metropolis, both in Akwa Ibom State, Nigeria. Soil sample (Sandy-loam) was obtained from Afa Ofot, Abak Road in Uyo Metropolis, Akwa Ibom State, Nigeria for soil analysis. The study was carried out in the Laboratory and Green House of the Department of Botany, University of Calabar, Calabar, Cross River State, Nigeria.

Source of fungus and morphological identification

The fungus used in this research work was isolated from spent engine oil contaminated soil collected from the Uyo Mechanic Village, Uyo Metropolis, Akwa Ibom State, Nigeria using Direct Plate Method (DPM). Approximately 2g of the spent engine oil contaminated soil was placed on Sabouraud Dextrose Agar (SDA) in Petri dishes. Four (4) sections were inoculated per Petri dish. The plates were incubated at 28 ± 1°C until fungus growth was noticed. After 5 days, the isolate was sub-cultured on freshly prepared SDA to obtain pure culture. Isolated fungus was microscopically (Olympus optical, Phillipines) identified as far as possible using the identification guides of the International Mycological Institute, Kew [14].

In vitro assay

2ml of spent and unspent engine oil was first poured into different Petri dishes (90mm) using sterile syringe, and with a sterilized No.2 cork borer of 5.5mm in diameter, a disc of the matured culture was punched out and inoculated at the centre of plates and incubated at room temperature of (28±1oC) for 7days. As a control, the fungus was inoculated on Potato Dextrose Agar instead of spent and unspent engine oil. Three (3) control plates were prepared for each sample. Measurement of the mycelium growth diameter was obtained daily for seven days using a calliper and metre rule [15].

In vivo assay

Soil analysis

Sandy-loam soil was collected and analysed at the Research Laboratory of the Department of Soil Science, University of Calabar, Calabar, Cross River State, Nigeria for percentage moisture, pH, total Nitrogen N (determined using Kjedahl's method followed by spectrophotometry procedure), organic carbon (determined by oxidation with $K_2Cr_2O_7$ [16], Available phosphorus P, calcium Ca, and magnesium Mg (determined using the method of [17], Potassium K (determined using flame photometry).

Soil sterilization

Soil sterilization was conducted in the Department of Botany green house, University of Calabar, Nigeria under mean temperature of 27˚C. The top soil collected at 0-45cm depth were heat sterilized in a cut covered metal drum using firewood at 100˚C for 20 minutes and allowed to cool. The sterilized soil was dispensed into polyethylene bags.

Soil treatment/Soil inoculation

Polyethylene bags were filled with about 5 kilogram (5kg) of the sandy-loam soil treated with 20, 40, 60, 80 and 100ml concentration of spent engine oil. The treatments were replicated thrice and laid out (Experimental Design) in a Complete Randomized Design (CRD). Soil inoculation was carried out using the methods of [18]. 180mm (2 Petri dishes) containing seven day old mycelium of *Penicillium sp*. grown on PDA was dissolved in 100ml of distilled water and inoculated into the soil treated with 20ml of spent engine oil, while 360mm (4 Petri dishes) was dissolved in 100ml of distilled water and inoculated into the soil treated with 40ml of spent engine oil. Soil treated with 60ml of spent engine oil was inoculated with 540mm (6 Petri dishes) dissolved in 100ml of distilled water, while 80ml was inoculated with 720mm (8 Petri dishes) dissolved in 100ml of distilled water and 100ml treatment was inoculated with 900mm (10 Petri dishes) of seven day old mycelium of *Penicillium sp*. dissolved in 100ml of distilled water. Soil, treatment (spent engine oil) and mycelium of *Penicillium sp*. were thoroughly mixed before planting with *Telfairia occidentalis* seeds.

Planting of *T. occidentalis*

Three to four seeds of *T. occidentalis* were sown in polyethylene bags containing spent engine oil polluted soil and inoculated with mycelium of *Penicillium sp*. After seed emergence, the plant was reduced to two stands per bag. As the plants grew, growth parameters such as plant height (PH), leaf area (LA), and number of leaves (NL) was collected at 7 Days after Planting (DAP), 14 (DAP), 21 (DAP) and 28 (DAP). Fresh weight (FW) and Dry weight (DW) were collected at 28 (DAP) in three replicates. Frequency of watering was morning and evening.

Statistical analysis

Data obtained in this research work were analysed by one way analysis of variance (ANOVA) using IBM SPSS ver. 21 and sample means were compared using Least Significant Difference (LSD) and Duncan multiple range test to obtain significant data.

Results

Isolated fungus

Penicillium sp. (Figure 1) was isolated from spent engine oil contaminated soil and used in this study.

***In vitro* bioremediation assay**

Results of *in vitro* bioremediation potentials of *Penicillium sp*. grown on spent and unspent engine oil carried out in this study is presented in (Table 1). Results show that the growth diameter of *Penicillium sp*. inoculated on spent and unspent engine oil was 1.12 ± 0.04cm, 3.46 ± 0.02cm, 3.57 ± 0.05cm, 3.67± 0.01cm, 3.69 ± 0.03cm, 4.08 ± 0.02cm, 4.50 ± 0.04cm and 0.81± 0.01cm, 2.14 ± 0.04cm, 2.47 ± 0.02cm, 3.31± 0.03cm, 4.07 ± 0.01cm, 4.50 ± 0.02cm and 4.50 ± 0.03cm on the first to seventh day of incubation respectively while that of the control (Untreated) was 1.29 ± 0.01cm, 2.46 ± 0.03cm, 3.37 ± 0.02cm, 3.49 ± 0.04cm, 3.53 ± 0.02cm, 3.63 ± 0.01cm and 3.73 ± 0.02cm on the first to seventh day of incubation respectively. Results therefore, showed that *Penicillium sp*. had a significant effect ($p \leq 0.05$) in degrading the hydrocarbons present in the spent and unspent engine oil relative to control after seven days observation period.

In vivo bioremediation assay

Soil analysis

Soil analysis revealed the presence of reasonable level of sand (20.2%), silt (51.2%), and Clay (22.4%) as well as macronutrients Potassium (K) 139mg/kg, Phosphorus (P) 63mg/kg. Nitrogen (N), organic carbon (C), Magnesium (Mg) and Calcium (Ca) was 38mg/kg, 1.90mg/kg, 136mg/kg and 107mg/kg respectively. The soil pH was 7.2 as presented in (Table 2).

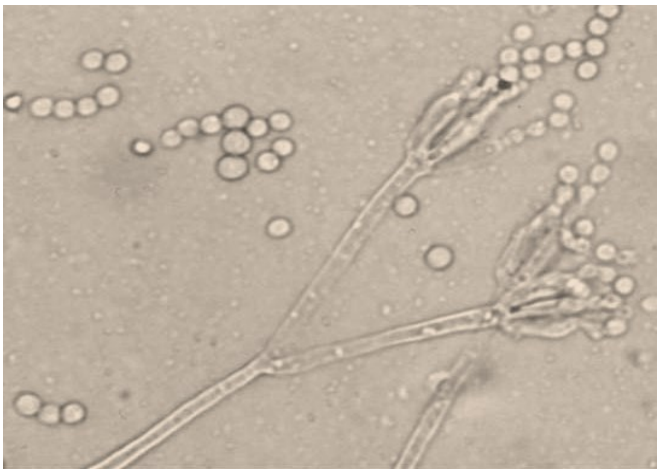

Fig. 1: Photomicrograph of *Penicillium sp*. × 40

Table 1: Growth diameter of *Penicillium sp*. grown on spent and unspent engine oil for seven days (cm)

Treatments	Days of incubation and mycelium growth (cm)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Spent engine oil	1.12±0.04	3.46±0.02	3.57±0.05	3.67±0.01	3.69±0.03	4.08±0.02	4.50±0.04
Unspent engine oil	0.81±0.01	2.14±0.04	2.47±0.02	3.31±0.03	4.07±0.01	4.50±0.02	4.50±0.03
Control	1.29±0.01	2.46±0.03	3.37±0.02	3.49±0.04	3.53±0.02	3.63±0.01	3.73±0.02
Least Significant Difference	0.98*						

Note: n = 3; Mean ± Standard Deviation

Table 2: Soil analysis

Soil constituents	content
Texture (%)	
Sand	20.2
Silt	51.2
Clay	22.4
Soil pH	
pH	7.2
Nutrients (mg/kg)	
Potassium (K)	139
Phosphorus (P)	63
Nitrogen (N)	38
Organic carbon (C)	1.90
Magnesium (Mg)	136
Calcium (Ca)	107

Bioremediation effect of *Penicillium sp*. on growth performance of *T. occidentalis* grown in spent engine oil contaminated soil at the different concentrations

Growth performance of *T. occidentalis* on spent engine oil contaminated soil at the different concentrations 0ml (control), 20, 40, 60, 80, and 100mls and treatment levels with mycelium of *Penicillium sp*. at 20ml/180mm, 40ml/360mm, 60ml/540mm, 80ml/720mm and 100ml/900mm for 7, 14, 21 and 28 (DAP) is given below.

Plant height (cm)

Results of the mean plant height of *T. occidentalis* grown on spent engine oil contaminated soil at the different concentrations inoculated with mycelium of *Penicillium sp*. is presented in (Table 3). Results showed that the mean plant height of the control (untreated) 0ml (12.2 ± 0.5) 7 DAP was not significantly greater ($p<0.05$) than those means for plant grown in spent engine oil contaminated soil at 20/180 (13.8 ± 0.04), 40/360 (13.7 ±0.03), 60/540 (14.7 ± 0.03), 80/720 (13.3 ± 0.01) and 100ml/900mm (14.5 ± 0.02) concentrations/treatments as presented in (Table 3). Results therefore, showed that there was no progressive reduction in plant height of *T. occidentalis* as the concentration of the spent engine oil increased from 20 to 100ml. At 14 DAP; the mean plant height of the control 0ml (17.6 ± 0.02) was also not significantly greater than those means for plant grown in soil at 20/180, 40/360, 60/520 and 80ml/720mm concentrations/treatments except at 100ml/900mm (15.5 ± 0.02) concentration/treatment. However, retardation of growth was not observed at this treatment level. At 21 DAP, it was observed that the mean plant height of the control 0ml (20.7 ±0.1) was also not significantly greater ($p<0.05$) than those plant grown in 20 to 80ml concentrations except at100ml/900mm (13.5 ± 0.02) concentration/treatment. At 28 DAP, it was observed that the soil became very hard and no trace of spent engine oil was observed on the surface of the soil. At 28 DAP, the growth of *T. occidentalis* initially retarded at 100ml concentration 21 DAP was progressive. This shows that the *Penicillium sp*. was able biodegrade the hydrocarbons which initially created a hydrophobic environment limiting water absorption through the roots. Also at 28 DAP, there was no toxic effect observed on the *T. occidentalis* as a result of treatment with spent engine oil, rather the *T. occidentalis* showed reasonable growth level at the different concentration/treatment levels as compared with the control. The plant began producing pods 61 DAP.

Table 3: Mean plant height of *T. occidentalis* grown in spent engine oil contaminated soil inoculated with mycelium of *Penicillium sp*.

Concentration (ml)/treatment (mm)	0(control)	20/180	40/360	60 /540	80/720	100/900
Plant height (cm)						
7 DAP	12.2 ± 0.5^{a}	13.8 ± 0.04^{a}	13.1 ± 0.04^{a}	14.7 ± 0.03^{a}	13.5 ± 0.01^{a}	14.5 ± 0.02^{a}
14 DAP	17.6 ± 0.02^{b}	15.7 ± 0.03^{a}	15.6 ± 0.02^{a}	17.4 ± 0.2^{b}	14.1 ± 0.02^{a}	15.5 ± 0.02^{b}
21 DAP	20.7 ± 0.1^{c}	19.1 ± 0.02^{b}	19.1 ± 0.04^{b}	18.2 ± 0.03^{c}	$18.\ 8 \pm 0.1^{b}$	13.5 ± 0.02^{a}
28 DAP	22.8 ± 0.2^{d}	23.2 ± 0.1^{c}	21.2 ± 0.2^{c}	20.1 ± 0.01^{d}	19.1 ± 0.04^{c}	18.2 ± 0.5^{c}

Note: (a, b, c and d are subscript), Values with same subscript in the same column are not significantly different, but values with different subscript in the same column are significantly different at ($p<0.05$)

Leaf area (cm)

Results of the mean leaf area of *T. occidentalis* grown on spent engine oil contaminated soil at the different concentrations inoculated with mycelium of *Penicillium sp*. is presented in (Table 4). Results showed that at 7 DAP, the mean leaf area of the *T. occidentalis* grown in spent engine oil contaminated soil at 20/180 (8.5 ± 0.02), 40/ 360 (9.3 ± 0.02), 60/ 540 (7.7 ± 0.02), 80/720 (8.3 ± 0.03) and 100ml/900mm (8.4 ± 0.001) concentrations/treatments was significantly greater ($p<0.05$) than the mean leaf area of the control 0ml (5.1 ± 0.2). At 7 DAP, The leaf area of *T. occidentalis*

was observed to increase tremendously as compared to the control (Table 4). At 14 DAP; the mean leaf area of the control 0ml (10.6 ± 0.1) was not significantly greater ($p<0.05$) than the mean leaf area of those plant grown on 20/180 (10.7 ± 0.01), 40/360 (11.7 ± 0.03), 60/540 (10.3 ± 0.01), 80/720 (10.1 ± 0.02) and 100ml/900mm (10.8 ± 0.03) concentrations/treatment of spent engine oil contaminated soil. At 21 DAP; there was also no significant difference observed between the mean leaf area of the control and those plants grown on spent engine contaminated soil at the different concentrations/treatments. Leaf curl was however observed on some of the plant at higher concentrations of 80 and 100mls. At 28 DAP, the leaf curl initially observed at 21 DAP was no longer visible. There was also no significant difference ($p<0.05$) between the control 0ml (20.1 ± 0.02) and the plant grown on 20/180 (19.2 ± 0.1), 40/360 (18.2 ± 0.05), 60/520 (18.1 ± 0.02), 80/720 (18.2 ± 0.03) and 100ml/900mm (17.9 ± 0.02) concentrations/treatments. Results therefore, showed that there was a reasonable increase in the mean leaf area of *T. occidentalis* as compared to the control.

Table 4: Mean leaf area of *T. occidentalis* grown in spent engine oil contaminated soil inoculated with mycelium of *Penicillium sp*.

Concentration (ml)/treatment (mm)	0(control)	20/180	40/360	60 /540	80/720	100/900
Leaf area (cm)						
7 DAP	5.1 ± 0.2^{a}	8.5 ± 0.02^{a}	9.3 ± 0.02^{a}	7.7 ± 0.02^{a}	8.3 ± 0.03^{a}	8.4 ± 0.01^{a}
14 DAP	10.6 ± 0.1^{b}	10.7 ± 0.01^{a}	11.7 ± 0.03^{b}	17.4 ± 0.2^{b}	10.1 ± 0.02^{a}	15.5 ± 0.02^{b}
21 DAP	15.0 ± 0.03^{c}	15.8 ± 0.03^{b}	19.1 ± 0.04^{c}	14.1 ± 0.03^{c}	13.6 ± 0.02^{b}	13.5 ± 0.02^{c}
28 DAP	20.1 ± 0.02^{d}	19.2 ± 0.1^{c}	21.2 ± 0.2^{d}	18.2 ± 0.01^{d}	18.2 ± 0.03^{c}	17.9 ± 0.5^{d}

Note: (a, b, c and d are subscript), Values with same subscript in the same column are not significantly different, but values with different subscript in the same column are significantly different at ($p<0.05$)

Leaf count (Number of leaves)

Results of the mean leaf count of *T. occidentalis* grown in spent engine oil contaminated soil at the different concentrations inoculated with mycelium of *Penicillium sp*. is presented in (Table 5). Results showed that at 7 DAP, the mean leaf count of the *T. occidentalis* grown in spent engine oil contaminated soil at 20/180 (6.0 ± 0.03), 40/360 (6.0 ± 0.02), 60/540 (6.0 ± 0.02) and 80ml/720mm (7.0 ± 0.02) concentrations/treatments was significantly greater ($p<0.05$) than the mean leaf count of the control 0ml (5.1 ± 0.2) except at 100ml/900mm 4.0 ± 0.02 concentration/treatment. At 14 DAP; the mean leaf count of the control 0ml (11.0 ± 0.02) was not significantly higher than those grown in 20/180 (10.7 ± 005), 40/360 (10.5 ± 0.1), 60/540 (10.1 ± 0.01) and 80ml/720mm (10.2 ± 0.02) except at 100ml/900mm (8.2 ± 0.03) concentration/treatment. However, at 21 DAP number of mean leaf count of the control 0ml (16.5 ± 0.1) was not significantly higher ($p<0.05$) than those plant grown in spent engine oil contaminated soil at 20ml/180mm (16.0 ± 0.2), 40ml/360mm (15.0 ± 0.03), 60ml/520mm (15.0 ± 0.5), 80ml/720mm (15.3 ± 0.02) and 100ml/900mm (15.0 ± 0.01) concentrations/treatments. At 28 DAP, number of mean leaf count of the control 0ml (18.2 ± 0.1) was not significantly higher than those grown in 20/180 (18.1 ± 0.03), 40/360 (17.1 ±0.02), 60/520 (17.7 ± 0.2), and 80ml/720mm (17.2 ± 0.1) concentrations/treatments except at 100ml/900mm (15.0 ± 0.01) concentration/treatment level. It is noteworthy to state that at 28 DAP, the leaves of *T. occidentalis* were observed to be evergreen, this confirms that *Penicillium sp*. was able to biodegrade the hydrocarbons present in the spent engine oil contaminated soil and as such *T. occidentalis* was able to combat stomata and transpiration problems.

Table 5: Mean leaf count of *T. occidentalis* grown in spent engine oil contaminated soil inoculated with mycelium of *Penicillium sp*.

Concentration (ml)/treatment (mm)	0(control)	20/180	40/360	60 /540	80/720	100/900
Leaf count (Number of leaves)						
7 DAP	5.0 ± 0.2^{a}	6.0 ± 0.03^{a}	6.0 ± 0.02^{a}	6.0 ± 0.02^{a}	7.0 ± 0.02^{a}	4.0 ± 0.02^{a}
14 DAP	11.9 ± 0.02^{b}	10.7 ± 0.05^{b}	10.5 ± 0.1^{b}	10.0 ± 0.01^{b}	10.2 ± 0.02^{b}	8.2 ± 0.03^{b}
21 DAP	16.5 ± 0.1^{c}	16.0 ± 0.2^{c}	15.0 ± 0.03^{c}	15.0 ± 0.5^{c}	$14.\ 3 \pm 0.02^{c}$	15.0 ± 0.01^{c}
28 DAP	18.2 ± 0.1^{d}	18.1 ± 0.03^{d}	17.1 ± 0.02^{d}	17.7 ± 0.2^{d}	17.2 ± 0.1^{c}	15.0 ± 0.01^{d}

Note: (a, b, c and d are subscript), Values with same subscript in the same column are not significantly different, but values with different subscript in the same column are significantly different at ($p<0.05$)

Fresh weight (g) and Dry weight (g)

Results of the mean fresh weight (g) and dry weight (g) of *T. occidentalis* grown in spent engine contaminated soil inoculated with mycelium of *Penicillium sp*. obtained 28 DAP is presented in (Table 6). At 28 DAP, mean Fresh weight (FW) (g) of the control 0ml (3.60 ± 0.01) was not significantly higher ($p<0.05$) than those that were grown in spent engine contaminated soil and treated with mycelium of *Penicillium sp*. at 20/180 (3.56 ± 0.01), 40/360 (3.44 ± 0.01) and 60ml/540mm (3.43 ± 0.0)1 concentrations/treatments except at 80ml/720mm (3.30 ± 0.01) and 100ml/900mm (3.21 ± 0.01) concentration/treatment. There was however, not much progressive reduction in plant fresh weight as the concentrations/treatments of the spent engine oil increased from 20 to 100ml and 180mm to 900mm respectively. At 28 DAP, mean Dry weight (DW) (g) of the control 0ml (2.1 ± 0.01) was also not significantly higher ($p<0.05$) than those plant grown on spent engine oil contaminated soil and treated with mycelium of *Penicillium sp*. at 20/180 (2.1 ± 0.01), 40/360 (2.1 ± 0.01), 60/540 (1.9 ± 0.01) and 80ml/720mm (1.9 ± 0.01) concentrations/treatments except at 100ml/900mm (1.5 ± 0.01) concentration/treatment. Increase in the concentration of the spent engine oil slightly reduced the fresh and dry weight of *T. occidentalis* at the higher concentrations/treatments but showed no significant difference as compared with the control.

Table 6: Mean fresh and dry weight (g) of *T. occidentalis* grown in spent engine oil contaminated soil inoculated with mycelium of *Penicillium sp*.

Concentration (ml)/treatment (mm)	FW (g)	DW (g)
0 (control)	3.60 ± 0.01^{a}	2.1 ± 0.01^{a}
20/180	3.56 ± 0.01^{a}	2.1 ± 0.01^{a}
40/360	3.44 ± 0.01^{a}	2.1 ± 0.01^{a}
60/540	3.43 ± 0.01^{a}	1.9 ± 0.01^{a}
80/720	3.30 ± 0.01^{b}	1.9 ± 0.01^{a}
100/900	3.21 ± 0.01^{c}	1.5 ± 0.01^{b}

Note: (a, b, c and d are subscript), Values with same subscript in the same column are not significantly different, but values with different subscript in the same column are significantly different at ($p<0.05$)

Discussion

Spent engine oil contaminated soil is a major factor limiting the growth and yield of crops and as such effective management is critical for the profitable production of crops. In this study *Penicillium sp.* isolated from spent engine oil contaminated soil obtained at the Uyo Mechanic village in Afa ofot in Uyo Metropolis of Akwa Ibom State, Nigeria was studied for its ability to bio-remediate spent engine oil contaminated soil at different concentrations both *in vitro* and *in vivo*.

Authors like Mandri and Lin [19], Quinones-Aquilar et al. [20] and Bouchez et al. [21] have reported on fungi that are able to degrade various pollutants while Yateem et al. [22], Juhasz and Naidu [23], Saraswathy and Hallberg [24], Adekunle et al. [25], Atagana et al. [26], Husaini et al. [27], Gesinde et al. [28], Obire and Anyanwu [29], and Hadibarata and Tachibana [30] studied the biodegradation of petroleum products by fungi which is in conformity with this study. Soil borne fungi such as *Penicillium sp*. has been reported to produce extracellular enzymes which breakdown complex carbohydrates and as such make possible the degradation of various pollutants. Romero et al. [31] reported the ability of *Penicillium sp*. to remediate pollutants in the presence of salt which is a useful biological treatment without damage to the physically sensitive ecosystem. *Penicillium sp*. was used in this study to test its ability to bio-remediate spent engine oil polluted soil both *in vitro* and *in vivo*. Results of the *in vitro* bioremediation assay showed a significant increase ($p<0.05$) in the mycelia growth of *Penicillium sp.* relative to the control (Table 2) when inoculated on spent and unspent engine oil and incubated for seven days. This finding is in conformity with that of Vanishree et al. [32] who reported on the biodegradation of petrol using *Penicillium sp*. The increase rates of mycelia growth of *Penicillium sp.* fungus on spent and unspent engine oil in this study might have been due to the fact that the fungus utilized spent engine oil as a medium for its growth using extracellular enzymes which agrees with the work of Bartha and Atlas [33]. Researchers like Singh [34] listed some genera of fungi that were isolated from an oil polluted environment which had been demonstrated to contain members that can degrade petroleum hydrocarbons. Juhasz and Naidu [23], also mentioned some soil borne fungi such as *Aspergillus* and *Penicillium* which were found to be potential degraders of crude oil hydrocarbons while researchers like Ryan et al. [35] and Srivastava and Thakur [36] reported *Fusarium solani*, *Fusarium oxysporum*, *Trichoderma viride* and *Aspergillus niger* grown in acidic medium which also showed good growth respectively.

In this study, results of the *in vivo* bioremediation assay using *Penicillium sp*. mycelium treated spent engine oil contaminated soil at different concentrations/treatment levels of 20/180, 40/360, 60/540, 80/720 and 100ml/900mm showed that the spent engine oil had no significant effect ($p<0.05$) on the growth performance (plant height, leaf area, leaf count (number of leaves) of *T. occidentalis* at 7, 14, 21 and 28 DAP and on fresh and dry weight 28 DAP when compared with the control (0ml) (Tables 3-6). The observed effect of *Penicillium sp*. treated spent engine oil contaminated soil on the growth performance of *T. occidentalis* agrees with the findings of other researchers like Adekunle et al. [25] that strains of the genus *Penicillium* are good hydrocarbon assimilators and that there have the ability to transform xenobiotics compounds like phenol into less mutagenic products. Workers like Pedro et al. [37] and Abdusalam et al. [38] also reported that *Penicillium sp* has the ability to degrade monocyclic aromatic hydro carbons such as benzene, toluene, ethyl benzene and xylene; BTEX), phenol compounds and heavy metals like lead, nickel and iron using mono-oxygenases, forming a trans-diol.

Conclusion

This study showed that *Penicillium sp*. a soil borne fungus can biodegrade hydrocarbons present in spent engine oil and as such is a good tool for bioremediation.

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