Bioremediation potential of mycorrhiza fungi in crude oil contaminated soil planted with *Costus lucanusianus*

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Abstract

This study was conducted to assess the impact of arbuscular mycorrhizal fungi on the performance of *Costus lucanusianus* plant under crude oil contaminated soil. About 10 kg sterilized soil was contaminated with bonny light crude oil at levels. *Costus lucanusianus* was planted on the soil by stem cutting and inoculations in treatment with AM consist of 20 g of *Glomus clarum*. Data on residual TPH content of the soil, plant height, number of leaves, fresh and dry weights and percent mycorrhiza root colonization were collected at 4, 8 and 12 weeks after planting (WAP). The 2 x 4 factorial experiment was laid out in completely randomized design (CRD). The results showed that AM inoculated treatments recorded higher and significantly (p<0.05) different plant height, number of leaves, fresh and dry weight. The TPH degradation and removal was higher with treatments inoculated with AM compared to non AM inoculated treatments. This Am colonization resulted in improved physiological parameters of *costus* plant.

Key-words: Arbuscular Mycorrhiza, *Costus lucanusianus*, Bioremediation, Bonny Light Crude Oil, Contaminated Soil

Introduction

Crude oil, a type of petroleum hydrocarbons, comprises various proportions of alkanes (e.g., methane, ethane and propane), aromatics (e.g. benzene, toluene, ethylbenzene and xylene, collectively known as BTEX) and polycyclic aromatic hydrocarbons (PAHs; e.g. naphthalene, phenanthrene, anthracene, benzo[a]pyrene) (Lyons, 1996; Frick et al., 1999; Adipah, 2019). The over dependence on petroleum hydrocarbons as a major source of energy for homes and industries has led to the pollution of lands (particularly agricultural lands) due to oil spillage during exploration and processing operations. Crude oil contaminated soils are not suitable for agricultural and recreational use and are potential sources of surface and ground water contamination (Schwab et al., 1999; Adipah, 2019). Incineration, thermal desorption, soil washing, etc. are some of the physico-chemical techniques used to treat petroleum hydrocarbon polluted soil (Dadrasnia et al., 2015; Frick et al., 1999). However, these techniques are often expensive and have limited local application due to the complex scientific knowledge needed to operate the component units (Gong, 2012; Frick et al., 1999).
Biological treatments are better alternative methods for the treatment of contaminated soil. These methods are economically and eco-friendly and have shown promising results in the treatment of organic compounds contaminated soil, especially petroleum hydrocarbons (Graj et al., 2013; Soleimani et al., 2010). Mycorrhiza, an association between plants and fungi that colonize the cortical tissue of roots during the active periods of the plant growth have been suggested to improve biodegradation of recalcitrant organic pollutants due to the large size and very high surface interface with soil (Sharma et al., 2007).

Studies have shown that mycorrhizal fungi in bioremediation receive a direct supply of carbon from their plant hosts to support growth into contaminated substrates. Some of this carbon may subsequently be available to bacteria associated with the mycorrhizal mycelium (Sun et al., 1999) and these may have implications for bioremediation in the mycorrhizosphere. Several studies have also suggested that Mycorrhiza fungi species collaborate with some of these microorganisms in phytoremediation process (Alarcon et al., 2008; Liu and Dalpe, 2009; Teng et al., 2010).

The study therefore sought to: 1) determine the remediation potential of mycorrhiza fungi in crude oil contaminated soil planted with African spiral ginger (Costus lucanusianus) and 2) determine the effect of mycorrhiza fungi in enhancing the survival and growth of African spiral ginger (Costus lucanusianus) in crude oil contaminated soil.

**Material and methods**

**Experimental Site**

The study was carried out during the growing seasons of 2013, 2014 and 2015 at the Department of Botany Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State.

**Planting Materials**

The arbuscular mycorrhiza fungi used for the study was sourced from the Soil Microbiology Laboratory, Department of Agronomy, University of Ibadan, Ibadan, Oyo State while Bonny Light Crude oil was obtained from Nigerian National Petroleum Corporation (NNPC), Port Harcourt refinery, Rivers State. African spiral ginger was sourced from the Department of Botany Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State.

**Soil Preparation**

Soil samples at depth 0 – 15 cm were collected from the Department of Botany Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State. The soil samples were air dried, sieved through a 2 mm sieve, sterilized and filled in 10 kg capacity pot.

**Planting and Management**

The crude oil (200, 300 and 500 mls/pot) was mixed thoroughly with the soil and allowed to stand for two weeks. After two weeks, African spiral ginger was planted by stem cuttings (2.5 cm long). Inoculation in treatments containing Glomus clarum consisted of 20 g of root-soil-fungal mixture blended into the central third of the soil at planting. (Carling et al., 1978). Hand weeding was done as at when due.

**Data Collection**

Plant height, number of leaves, fresh and dry weights were determined at 4, 8 and 12 weeks after planting (WAP). The plant height was measured in centimeter and determined with the aid of a meter rule. The number of leaves was done through visual counting. The fresh biomass yield was determined by cutting fresh biomass and weighed while for the dry weight the fresh biomass was oven-dried to a constant weight at a temperature of 65ºC. The total petroleum hydrocarbon content of the soil was also determined at 4, 8 and 12 weeks after planting (WAP).

**Experimental Design**

The experimental design was completely randomized design (CRD) replicated three times. It was a factorial experiment with two factors- Mycorrhirzal application at two levels (with and without) and Crude oil contaminant at 4 levels making a total of 8 treatment combinations. The treatments are:

**Factors:**

(a) Mycorrhizal inocula

i. Glomus clarum inoculation

ii. Without Glomus clarum inoculation

(b) Crude oil (Bonny Light) contaminant

i. No crude oil
ii. 20 mls/kg soil; 200 mls/pot  
iii. 30 mls/kg soil; 300 mls/pot  
iv. 50 mls/kg soil; 500 mls/pot.

**Determination of Physicochemical Properties of the Soil Sample**

Soil mechanical analysis was estimated by the hydrometer method of Bouyoucous (1951) while the soil pH was determined by the method of Udoh and Ogunwale (1986). The organic carbon was determined by the modified Walkley-Black procedure (Nelson and Sommers, 1996). The total nitrogen and available phosphorous content of the soil sample were determined by the micro Kjeldahl digestion and distillation method as described by Udo and Ogunwale (1986) and Bray P 1 method (Bray and Kurtz, 1945). The exchangeable bases (K, Na, Ca and Mg) were determined by the method of Jackson (1958); Ca and Mg were read off using atomic absorption spectrophotometer (AAS) while K and Na were read off using flame photometer. Similarly, the effective cation exchange capacity was estimated by the summation method of Juo et. al. (1976) while the exchangeable acidity was estimated by the method of Mclean (1982).

**Determination of Percentage Mycorrhizal Roots Colonization**

Percentage roots colonization was determined using the grid-line intersect method of Kormanik and McGraw (1982).

**Microbial enumeration of fungi**

Numbers of viable fungi were estimated by the plate count technique.

**Total petroleum hydrocarbon (TPH)**

Soxhlet extraction method was used for the total petroleum hydrocarbon (TPH) content. Analytical determinations of the hydrocarbons in the soil extracts were performed by infrared spectrophotometer (IR). Quantification of the total petroleum hydrocarbon (TPH) content was done using the procedure described by the USEPA SW-846 series method 3540.

After collection, the extract was passed through sodium sulfate and silica gel to remove water and polar constituents. An aliquot of the extract was then placed in the infrared spectrophotometry (IR) analyzer. The TPH value was determined by comparison to a three-point calibration curve constructed from dilutions of a stock solution of a 2:3:3 volume ratios of chloro- benzene, isoctane, and n-hexadecane made up in perchloroethylene (PCE) (US EPA, 2017).

**Statistical analysis**

All data collected were subjected to analysis of variance (ANOVA) by Proc. GLM of GenStat version 17, and significant means were separated using Duncan Multiple Range Test (DMRT).

**Results**

Table 1 shows the pre-treated soil characteristics with a pH of 4.9, the values of the major nutrient elements are as follows: total N: 0.10 %, available P: 28.70 mg/kg and exchangeable K: 0.12 cmol/kg. The textural class of the soil was sandy.

| Soil properties | Value |
|-----------------|-------|
| pH (H₂O)        | 4.9   |
| C (%)           | 1.24  |
| N (%)           | 0.10  |
| Av. P (mg/kg)   | 28.70 |
| Moisture content (%) | 7.8   |
| Exchangeable cations (cmol/kg) |  |
| Ca              | 6.0   |
| Mg              | 1.2   |
| K               | 0.12  |
| Na              | 0.08  |
| Al              | 0.16  |
| H⁺              | 0.82  |
| ECEC (cmol/kg)  | 8.4   |
| Particle size (g/kg) |  |
| Sand            | 840   |
| Silt            | 110   |
| Clay            | 50    |

| Textural class | Sandy |

**Effect of Mycorrhiza and Crude Oil on the Plant Height, Number of Leaves, Fresh weight and Dry weight of Costus at Different Weeks After Planting (WAP) Under Pot Experiment 1**

At all the weeks (except at 4 weeks after planting (WAP)) evaluated, mycorrhizal inoculated *costus* plants had significantly higher plant heights compared to non-mycorrhizal inoculated *costus* plants (Table 2).
Table 2: Effect of mycorrhiza and crude oil on the plant height, number of leaves, fresh and dry weights of *costus* at different weeks after planting (WAP) under pot experiment 1.

| Treatments | Plant height (cm) | Number of leaves | | | | | |
|------------|------------------|-----------------|---|---|---|---|---|---|---|---|---|
|            | 4WAP | 8WAP | 12WAP | 4WAP | 8WAP | 12WAP |
| Mycorrhizal | | | | | | | |
| M+         | 32.92 | 44.16 | 58.29 | 18.94 | 34.14 | 47.14 |
| M-         | 32.49 | 41.72 | 55.00 | 16.03 | 28.97 | 42.39 |
| LSD (0.05) | ns   | 1.25  | 1.55  | 0.91  | 1.12  | 1.18  |
| SE ()      | 0.36 | 0.44  | 0.54  | 0.32  | 0.39  | 0.42  |
| Crude oil (ml/pot) | | | | | | | |
| C0         | 41.97 | 56.80 | 73.48 | 21.67 | 41.11 | 57.72 |
| C1         | 29.94 | 41.02 | 54.05 | 17.50 | 28.97 | 42.39 |
| C2         | 32.88 | 40.95 | 56.66 | 18.50 | 27.78 | 37.89 |
| C3         | 26.04 | 32.99 | 44.39 | 12.28 | 25.11 | 37.67 |
| LSD (0.05) | 1.43 | 1.76  | 2.19  | 1.30  | 1.59  | 1.68  |
| SE ()      | 0.50 | 0.62  | 0.77  | 0.45  | 0.56  | 0.59  |

| Treatments | Fresh weight (g) | Dry weight (g) | | | | | |
|------------|-----------------|----------------|---|---|---|---|---|---|---|---|---|
|            | 4WAP | 8WAP | 12WAP | 4WAP | 8WAP | 12WAP |
| Mycorrhizal | | | | | | | |
| M+         | 46.02 | 56.11 | 83.64 | 24.56 | 28.51 | 42.34 |
| M-         | 44.24 | 54.68 | 82.50 | 22.37 | 26.31 | 39.65 |
| LSD (0.05) | 1.19 | 1.19  | ns   | 0.62 | 0.72  | 0.76  |
| SE ()      | 0.42 | 0.42  | 0.52  | 0.22 | 0.25  | 0.27  |
| Crude oil (ml/pot) | | | | | | | |
| C0         | 54.48 | 63.79 | 115.33 | 28.33 | 31.25 | 55.92 |
| C1         | 48.16 | 59.37 | 88.50 | 25.66 | 30.10 | 44.20 |
| C2         | 39.33 | 49.49 | 65.70 | 20.89 | 25.34 | 32.16 |
| C3         | 38.55 | 48.93 | 62.74 | 18.98 | 22.97 | 31.70 |
| LSD (0.05) | 1.69 | 1.68  | 2.10  | 0.88 | 1.02  | 1.07  |
| SE ()      | 0.59 | 0.59  | 0.73  | 0.31 | 0.34  | 0.38  |

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls of crude oil; C2 = 300 mls of crude oil; C3 = 500 mls of crude oil; LSD = Least significant difference; ns = not significant.

Crude oil application at 0 ml/pot resulted in significantly higher *costus* plant heights at 4, 8 and 12 WAP compared to the other application levels (Table 2) while 500 mls/pot crude oil had significantly lower values compared to those of 200 and 300 mls/pot crude oil, however at 8 and 12 WAP, 200 and 300 mls/pot crude oil were not significantly different.

Mycorrhizal inoculation resulted in significantly higher number of *costus* leaves at 4, 8 and 12 WAP compared to non-mycorrhizal inoculation (Table 2).

At 4, 8 and 12 WAP, treatment with 0 ml/pot crude oil resulted in significantly higher number of leaves of *costus* plant compared to the other treatments (Table 2) while treatment with 500 mls/pot crude oil had significantly lower number of leaves compared to those of 200 and 300 mls/pot crude oil but at 12 WAP, 500 and 300 mls/pot crude oil were not significantly different.

At all the weeks (except at 12 WAP) evaluated, mycorrhizal inoculation resulted in significantly higher fresh weight of *costus* plant compared to non-mycorrhizal inoculation (Table 2).

Treatment with 0 ml/pot crude oil at 4, 8 and 12 WAP had significantly higher values of *costus* fresh weights compared to the other treatments (Table 2). Similarly, treatment with 500 mls/pot crude oil resulted in significantly lower fresh weights of *costus* compared to that of 300 mls/pot crude oil, however at 4 and 8 WAP, 500 and 300 mls/pot crude oil were not significantly different.
Mycorrhizal inoculation resulted in significantly higher dry weight of *costus* plant at 4, 8 and 12 WAP compared to non-mycorrhizal inoculation (Table 2).

At all the weeks (4, 8 and 12 WAP) evaluated, significantly higher dry weights of *costus* were obtained from application with 0 ml/pot crude oil compared to the other application levels (Table 2) while application at 500 mls/pot crude oil resulted in significantly lower values compared to that of 300 mls/pot crude oil although at 12 WAP, 500 and 300 mls/pot crude oil were not significantly different. Similar trends were also observed under pot experiment 2 (Table 6).

| Treatments | Plant height (cm) | Number of leaves |
|------------|------------------|-----------------|
|            | 4WAP  | 8WAP  | 12WAP | 4WAP  | 8WAP  | 12WAP |
| Mycorrhizal |       |       |       |       |       |       |
| M+         | 20.28 | 30.92 | 50.66 | 14.81 | 28.67 | 41.08 |
| M-         | 26.60 | 36.16 | 43.79 | 10.39 | 21.53 | 32.00 |
| LSD (0.05) | 0.81  | 0.90  | 1.42  | 0.7   | 0.57  | 0.57  |
| SE ()      | 0.29  | 0.32  | 0.49  | 0.09  | 0.20  | 0.20  |
| Crude oil (ml/pot) |       |       |       |       |       |       |
| C0         | 28.69 | 43.48 | 62.37 | 15.33 | 32.33 | 47.11 |
| C1         | 25.61 | 32.94 | 45.48 | 13.33 | 25.78 | 35.70 |
| C2         | 20.41 | 31.54 | 45.47 | 12.78 | 21.61 | 30.83 |
| C3         | 19.06 | 26.19 | 35.60 | 8.44  | 20.67 | 30.72 |
| LSD (0.05) | 1.15  | 1.27  | 2.01  | 0.81  | 0.81  | 0.81  |
| SE ()      | 0.40  | 0.45  | 0.71  | 0.13  | 0.29  | 0.29  |

| Treatments | Fresh weight (g) | Dry weight (g) |
|------------|------------------|----------------|
|            | 4WAP  | 8WAP  | 12WAP | 4WAP  | 8WAP  | 12WAP |
| Mycorrhizal |       |       |       |       |       |       |
| M+         | 38.73 | 46.60 | 75.25 | 17.81 | 19.40 | 32.08 |
| M-         | 35.70 | 48.75 | 73.09 | 16.02 | 23.91 | 37.68 |
| LSD (0.05) | 0.43  | 1.82  | 1.53  | 0.74  | 0.69  | 0.74  |
| SE ()      | 0.43  | 0.64  | 0.54  | 0.26  | 0.24  | 0.26  |
| Crude oil (ml/pot) |       |       |       |       |       |       |
| C0         | 47.16a| 54.33a| 106.19a| 21.41 | 24.69 | 47.11 |
| C1         | 38.89b| 51.92a| 79.61b | 19.38 | 23.43 | 37.33 |
| C2         | 31.81c| 42.75b| 56.47c | 14.31 | 17.81 | 25.77 |
| C3         | 31.00c| 41.69b| 54.42c | 12.57 | 16.69 | 23.33 |
| LSD (0.05) | 0.60  | 2.57  | 2.16  | 1.04  | 0.98  | 1.05  |
| SE ()      | 0.60  | 0.90  | 0.76  | 0.37  | 0.34  | 0.37  |

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls of crude oil; C2 = 300 mls of crude oil; C3 = 500 mls of crude oil; LSD = Least significant difference; ns = not significant.

**Interaction of Mycorrhiza with Crude Oil on the Plant Height, Number of Leaves, Fresh weight and Dry weight of Costus at Different Weeks After Planting (WAP) Under Pot Experiment 1**

At 4 and 12 WAP, the combine use of 0 ml/pot crude oil with non-mycorrhiza inoculation had significantly higher plant height compared to the other treatment combinations (Table 3) but the combinations of 0 ml/pot crude oil with non-mycorrhiza inoculation and mycorrhiza with 0 ml/pot crude oil were not significantly different. The combine application of mycorrhiza with 0 ml/pot crude oil at 8 WAP resulted in significantly higher plant height compared to the other treatments although the interactions of mycorrhiza with 0 ml/pot crude oil and 0 ml/pot crude oil with non-mycorrhiza inoculation were not also not significantly different.
Significantly lower plant heights of *costus* plant across the weeks evaluated was obtained from the interaction of 500 mls/pot crude oil with non-mycorrhiza inoculation.

**Table 3**: Interactions of mycorrhiza with crude oil on the plant height, number of leaves, fresh and dry weights of *costus* at different weeks after planting (WAP) under pot experiment 1.

| Mycorrhiza | Crude oil (ml/pot) | Plant height (cm) | Number of leaves | Fresh weight (g) | Dry weight (g) |
|------------|--------------------|------------------|-----------------|-----------------|---------------|
|            | 4WAP | 8WAP | 12WAP | 4WAP | 8WAP | 12WAP | 4WAP | 8WAP | 12WAP |
| M+         | C0   | 41.86a | 56.87a | 72.20a | 24.00a | 42.56a | 62.00a |
| M+         | C1   | 30.51cd | 41.61bc | 52.53c | 19.22b | 36.11c | 47.22c |
| M+         | C2   | 32.19bc | 39.35d | 53.06bc | 17.78b | 29.89d | 39.78e |
| M+         | C3   | 27.12e | 35.60e | 42.19e | 13.00d | 28.00d | 39.56d |
| M-         | C0   | 42.08a | 56.72a | 74.76a | 19.56b | 36.67b | 53.44b |
| M-         | C1   | 29.37d | 40.43bc | 55.57bc | 19.33b | 28.33d | 44.33d |
| M-         | C2   | 33.57b | 42.55b | 56.25b | 15.44c | 25.67e | 36.00f |
| M-         | C3   | 24.95f | 30.39f | 46.58d | 11.56d | 22.22f | 35.78f |
| SE ()      | 0.71 | 0.88 | 1.09 | 0.64 | 0.74 | 0.83 |

Means with the same letter(s) along the column are not significantly different from each other at (p<0.05) using Duncan Multiple Range Test

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls of crude oil; C2 = 300 mls of crude oil; C3 = 500 mls of crude oil.

The interaction of mycorrhiza with 0 ml/pot crude oil at 4, 8 and 12 WAP resulted in significantly higher number of leaves of *costus* plant compared to the other treatment interactions (Table 3) while the combine application of 500 mls/pot crude oil with non-mycorrhiza had significantly lower number of leaves of *costus* plant.

The combination of mycorrhiza with 0 ml/pot crude oil at 4 and 8 WAP resulted in significantly higher *costus* fresh weights compared to the other treatments (Table 3) although at 8 WAP, the combinations of mycorrhiza with 0 and 200 mls/pot crude oil and 0 ml/pot crude oil with non-mycorrhiza inoculation were not significantly different. At 12 WAP, the combination of 0 ml/pot crude oil with non-mycorrhiza inoculation had significantly higher fresh weight of *costus* compared to the other treatments. Significantly lower fresh weights at 12 WAP was obtained from the combination of 500 mls/pot crude oil with non-mycorrhiza inoculation compared to the interaction of mycorrhiza with 500 mls/pot crude oil, however, the combinations of 500 mls/pot crude oil with non-mycorrhiza inoculation and of 300 mls/pot crude oil with non-mycorrhiza inoculation were not significantly different.

At 4 and 8 WAP, significantly higher dry weights of costus were obtained from the combine use of mycorrhiza with 0 ml/pot crude oil compared to the other treatment combinations (Table 3) though the combination of mycorrhiza with 0 ml/pot crude oil, mycorrhiza with 200 mls/pot crude oil and 0 ml/pot crude oil with non-mycorrhiza inoculation were not significantly different. At 12 WAP, interaction of 0 ml/pot crude oil with non-mycorrhiza inoculation had significantly higher dry weight compared to the other treatment combinations.
Significantly lower dry weights at 4 and 12 WAP resulted from the combine use of mycorrhiza with 500 mls/pot crude oil compared to mycorrhiza with 300 mls/pot crude oil although at 12 WAP, the combinations of mycorrhiza with 500 mls/pot crude oil and 500 mls/pot crude oil with non-mycorrhiza inoculation were not significantly different. Significantly lower dry weight of costus plant at 8 WAP was obtained from the combination of 500 mls/pot crude oil with non-mycorrhiza inoculation compared to that of 300 mls/pot crude oil with non-mycorrhiza inoculation but the combinations of 500 mls/pot crude oil with non-mycorrhiza inoculation and mycorrhiza with 500 mls/pot crude oil were not significantly different. Similar trends were observed under pot experiment 2 (Table 7).

Table 7. Interaction of mycorrhiza with crude oil on the plant height, number of leaves, fresh and dry weights of *costus* at different weeks after planting (WAP) under pot experiment 2.

| Mycorrhizal | Crude oil (ml/pot) | Plant height (cm) | Number of leaves |
|-------------|-------------------|-------------------|------------------|
|             |                   | 4WAP  | 8WAP  | 12WAP | 4WAP  | 8WAP  | 12WAP |
| M+          | C0                | 23.56c | 46.50a | 64.57a | 15.56b | 36.11a | 41.11b |
| M+          | C1                | 23.40c | 34.65c | 48.44c | 10.11e | 28.56c | 38.89c |
| M+          | C2                | 22.79c | 29.14e | 42.16d | 10.22f | 23.33e | 33.44d |
| M+          | C3                | 17.36e | 22.83f | 40.87d | 6.67g  | 25.00d | 28.00e |
| M-          | C0                | 33.82a | 40.46b | 60.16b | 18.57a | 30.22b | 55.38a |
| M-          | C1                | 28.42b | 33.93c | 48.78c | 14.89c | 18.00g | 34.44d |
| M-          | C2                | 20.77d | 31.23d | 42.52d | 13.11d | 21.33f | 33.89d |
| M-          | C3                | 17.42e | 29.55de| 30.33e | 10.67ef| 18.22g | 27.22e |
| SE ()       |                   | 0.57  | 0.63  | 0.99  | 0.19  | 0.40  | 0.40  |

Means with the same letter(s) along the column are not significantly different from each other at (p< 0.05) using Duncan Multiple Range Test

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls of crude oil; C2 = 300 mls of crude oil; C3 = 500 mls of crude oil.
Effect of Mycorrhiza and Crude Oil on the Total Petroleum Hydrocarbon (TPH) Content of Soil Planted with Costus at Different Weeks After Planting (WAP) Under Pot Experiment 1

Mycorrhizal inoculated plant at 4 and 12 WAP had significantly lower total petroleum hydrocarbon (TPH) content of the soil compared to the non-mycorrhizal inoculated *costus* plant (Table 4) whereas at 8 WAP, there was no significant difference with reference to mycorrhiza inoculation.

Table 4. Effect of mycorrhiza and crude oil on the total petroleum hydrocarbon (TPH) content of soil planted with *costus* at different weeks after planting (WAP) under pot experiment 1.

| Treatments           | Total petroleum hydrocarbon (mg/kg) |
|----------------------|------------------------------------|
|                      | 4WAP  | 8WAP  | 12WAP |
| Mycorrhizal          |       |       |       |
| M+                   | 65.75 | 57.94 | 52.68 |
| M-                   | 67.42 | 58.33 | 53.84 |
| LSD (0.05)           | 0.27  | ns    | 1.35  |
| SE ()                | 0.09  | 0.29  | 0.47  |
| Crude oil (ml/pot)   |       |       |       |
| C0                   | BDL   | BDL   | BDL   |
| C1                   | 86.29 | 73.21 | 68.93 |
| C2                   | 89.33 | 76.33 | 69.71 |
| C3                   | 90.71 | 83.00 | 74.40 |
| LSD (0.05)           | 0.38  | 1.15  | 1.91  |
| SE ()                | 0.13  | 0.41  | 0.67  |

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300 mls; C3 = 500 mls; LSD (0.05) = Least significant difference, BDL = below detection limit.

The application of 200 mls/pot crude oil resulted in significantly lower TPH content of the contaminated soil throughout the weeks (4, 8 and 12 WAP) evaluated compared to the other application levels (Table 4). However at 12 WAP, application at 200 and 300 mls/pot crude oil were not significantly different. Application at 500 mls/pot crude oil had significantly higher TPH content of the contaminated soil compared to that of 300 mls/pot crude oil. The TPH content of the soil under 0 ml/pot crude oil was below detection limit (BDL). Similar trend was observed under pot experiment 2 (Table 8).

Table 8. Effect of mycorrhiza and crude oil on the total petroleum hydrocarbon (TPH) content of soil planted with *costus* at different weeks after planting (WAP) under pot experiment 2.

| Treatments           | Total petroleum hydrocarbon (mg/kg) |
|----------------------|------------------------------------|
|                      | 4WAP  | 8WAP  | 12WAP |
| Mycorrhizal          |       |       |       |
| M+                   | 55.69 | 48.09 | 42.65 |
| M-                   | 57.07 | 49.50 | 43.03 |
| LSD (0.05)           | 0.40  | 1.06  | ns    |
| SE ()                | 0.14  | 0.37  | 0.49  |
| Crude oil (ml/pot)   |       |       |       |
| C0                   | BDL   | BDL   | BDL   |
| C1                   | 72.85 | 61.74 | 55.24 |
| C2                   | 75.67 | 64.43 | 55.25 |
| C3                   | 76.98 | 69.02 | 60.86 |
| LSD (0.05)           | 0.57  | 1.49  | 1.96  |
| SE ()                | 0.20  | 0.53  | 0.69  |

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300 mls; C3 = 500 mls; LSD (0.05) = Least significant difference, BDL = below detection limit.

Enteractions of Mycorrhiza with Crude Oil on the Total Petroleum Hydrocarbon (TPH) Content of Soil Planted with Costus at Different Weeks After Planting (WAP) Under Pot Experiment 1

The combination of mycorrhiza with 200 mls/pot crude oil at 4, 8 and 12 WAP had significantly lower TPH content of the contaminated soil planted with *costus* plant compared to the other treatment combinations (Table 5) however at 8 and 12 WAP, the combinations of 200 and 300 mls/pot crude oil with non-mycorrhiza inoculation and mycorrhiza inoculation with 200 and 300 mls/pot crude oil were not significantly different (Table 5).
Table 5. Interactions of mycorrhizal with crude oil on the total petroleum hydrocarbon (TPH) content of soil planted with *costus* at different weeks after planting (WAP) under pot experiment 1.

| Mycorrhizal | Crude oil (ml/pot) | Total petroleum hydrocarbon (mg/kg) |
|-------------|------------------|-----------------------------------|
|             |                  | 4WAP    | 8WAP    | 12WAP   |
| M+          | C0               | BDL     | BDL     | BDL     |
| M+          | C1               | 84.91e  | 72.60d  | 68.44b  |
| M+          | C2               | 88.19d  | 73.82d  | 68.57b  |
| M+          | C3               | 89.91c  | 81.02b  | 73.70a  |
| M-          | C0               | BDL     | BDL     | BDL     |
| M-          | C1               | 87.68d  | 76.93c  | 69.30a  |
| M-          | C2               | 90.48b  | 75.73c  | 70.97b  |
| M-          | C3               | 91.51a  | 84.98a  | 75.10a  |
| SE ()       |                  | 0.19    | 0.57    | 0.95    |

Means with the same letter(s) along the column are not significantly different from each other at (p< 0.05) using Duncan Multiple Range Test.

Similarly, the interaction of 500 mls/pot crude oil with non-mycorrhiza inoculation resulted in significantly higher TPH contents of the contaminated soil at 4 and 8 WAP compared to that of 300 mls/pot crude oil with non-mycorrhiza inoculation. Furthermore, interaction of 500 mls/pot crude oil with non-mycorrhiza inoculation at 12 WAP had significantly higher TPH content of the contaminated soil compared to the other treatment combinations but the interactions of 500 mls/pot crude oil with non-mycorrhiza inoculation and mycorrhiza inoculation with 500 mls/pot crude oil were not significantly different (Table 5). Similar trend was observed under pot experiment 2 (Table 9).

Table 9. Interactions of mycorrhizal with crude oil on the total petroleum hydrocarbon (TPH) content of soil planted with *costus* at different weeks after planting (WAP) under pot experiment 2.

| Mycorrhizal | Crude oil (ml/pot) | Total petroleum hydrocarbon (mg/kg) |
|-------------|------------------|-----------------------------------|
|             |                  | 4WAP    | 8WAP    | 12WAP   |
| M+          | C0               | BDL     | BDL     | BDL     |
| M+          | C1               | 71.41d  | 60.66d  | 54.42b  |
| M+          | C2               | 74.87c  | 64.08c  | 54.56b  |
| M+          | C3               | 76.47b  | 67.64b  | 60.09a  |
| M-          | C0               | BDL     | BDL     | BDL     |
| M-          | C1               | 74.29c  | 62.83c  | 55.93b  |
| M-          | C2               | 76.48b  | 64.79c  | 56.08b  |
| M-          | C3               | 77.50a  | 70.39a  | 61.62a  |
| SE ()       |                  | 0.28    | 0.74    | 0.98    |

Means with the same letter(s) along the column are not significantly different from each other at (p< 0.05) using Duncan Multiple Range Test.
Effect of mycorrhiza and crude oil on the mycorrhiza root colonization of *costus* at different weeks after planting (WAP) under pot experiment 1

At 4, 8 and 12 WAP, mycorrhiza inoculated plant had significantly higher mycorrhiza root colonization compared to the non-mycorrhiza inoculated plant (Table 10).

Table 10. Effect of mycorrhiza and crude oil on the mycorrhiza root colonization of *costus* at different weeks after planting (WAP) under pot experiment 1

| Treatments | Mycorrhiza root colonization (%) | 4WAP | 8WAP | 12WAP |
|------------|-----------------------------------|------|------|-------|
| Mycorrhizal|                                   |      |      |       |
| M+         | 49.32                             | 53.14| 58.80|
| M-         | 18.13                             | 20.14| 22.59|
| LSD (0.05) | 0.57                              | 0.62 | 0.62 |
| SE ()      | 0.20                              | 0.22 | 0.22 |
| Crude oil (ml/pot) |        |      |      |       |
| C0         | 41.45                             | 44.86| 48.64|
| C1         | 35.61                             | 38.29| 42.67|
| C2         | 31.74                             | 35.52| 39.88|
| C3         | 26.11                             | 27.88| 31.58|
| LSD (0.05) | 0.80                              | 0.88 | 0.87 |
| SE ()      | 0.28                              | 0.31 | 0.31 |

. M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300 mls; C3 = 500 mls; LSD (0.05) = Least significant difference

Across the weeks evaluated, treatments with 0 ml/pot crude oil had significantly higher mycorrhiza root colonization compared to the other treatments (Table 6) while application at 500 mls/pot crude oil resulted in significantly lower mycorrhiza root colonization compared to that of 300 mls/pot crude oil.

Table 11. Interaction of mycorrhiza with crude oil on the mycorrhiza root colonization of *costus* at different weeks after planting (WAP) under pot experiment 1

| Mycorrhizal | Crude oil (ml/pot) | Mycorrhiza root colonization (%) | 4WAP | 8WAP | 12WAP |
|------------|-------------------|----------------------------------|------|------|-------|
| M+         | C0                | 63.23a                           | 68.39a| 74.24a|
| M+         | C1                | 51.43b                           | 55.42b| 61.86b|
| M+         | C2                | 47.07c                           | 51.18c| 56.76c|
| M+         | C3                | 35.56d                           | 37.56d| 42.33d|
| M-         | C0                | 19.67e                           | 21.33e| 23.03e|
| M-         | C1                | 19.79e                           | 21.16e| 23.48e|
| M-         | C2                | 16.41f                           | 19.87f| 23.01e|
| M-         | C3                | 16.66f                           | 18.21g| 20.82f|
| SE ()      |                   | 0.39                             | 0.44 | 0.43 |

Means with the same letter(s) along the column are not significantly different from each other at (p < 0.05) using Duncan Multiple Range Test.

Enterraction of mycorrhiza with crude oil on the mycorrhiza root colonization of *costus* at different weeks after planting (WAP) under pot experiment 1

At 4, 8 and 12 WAP, the interaction of mycorrhiza with 0 ml/pot crude oil resulted in significantly higher mycorrhiza root colonization compared to the other treatment combinations (Table 11) while significantly lower mycorrhiza root colonization was obtained from the interaction of 500 mls/pot crude oil with non-mycorrhiza inoculation. Similar trends were observed under pot experiment 2 (Tables 12 and 13).
Table 12. Effect of mycorrhiza and crude oil on the mycorrhiza root colonization of *costus* at different weeks after planting (WAP) under pot experiment 2.

| Mycorrhizal | Mycorrhiza root colonization (%) | 4WAP | 8WAP | 12WAP |
|-------------|----------------------------------|------|------|-------|
| M+          |                                  | 46.63| 50.65| 56.91 |
| M-          |                                  | 17.78| 19.27| 21.54 |
| LSD (0.05)  |                                  | 0.49 | 0.62 | 0.63  |
| SE ()       |                                  | 0.17 | 0.22 | 0.22  |

Mycorrhizal root colonization (%): M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300 mls; C3 = 500 mls; LSD (0.05) = Least significant difference.

Table 13. Interaction of mycorrhiza with crude oil on the mycorrhiza root colonization of *costus* at different weeks after planting (WAP) under pot experiment 2.

| Mycorrhizal | Crude oil (ml/pot) | Mycorrhiza root colonization (%) | 4WAP | 8WAP | 12WAP |
|-------------|--------------------|----------------------------------|------|------|-------|
| M+          | C0                 | 57.69a                           | 62.60a| 69.02a|       |
| M+          | C1                 | 48.78b                           | 53.72b| 60.06b|       |
| M+          | C2                 | 45.31c                           | 49.46c| 56.26c|       |
| M+          | C3                 | 34.74d                           | 36.81d| 42.31d|       |
| M-          | C0                 | 18.47f                           | 19.48f| 20.78f|       |
| M-          | C1                 | 19.48e                           | 20.72e| 22.74d|       |
| M-          | C2                 | 16.23g                           | 18.14g| 20.07f|       |
| M-          | C3                 | 0.35                             | 0.44  | 0.44  |       |

Means with the same letter(s) along the column are not significantly different from each other at (p< 0.05) using Duncan Multiple Range Test.
Table 14 shows the effect of mycorrhiza and crude oil on the fungal total colony count (CFU/g soil) and the organisms identified in the experimental soil under pot experiment.

Table 14. Effect of mycorrhiza and crude oil on the fungal total colony count (CFU/g soil) and the organisms identified in the treated soil under pot experiment.

| Treatments | Fungal total colony count (cfu/g soil) | Fungi species identified |
|------------|---------------------------------------|-------------------------|
| M+C0       | 6x10^4                               | Aspergillus spp. Mucor spp. |
| M+C1       | 6x10^4                               | Aspergillus spp. Mucor spp. |
| M+C2       | 7x10^4                               | Aspergillus spp. Rhizopus spp. |
| M+C3       | 8x10^4                               | Aspergillus spp. Mucor spp. |
| M-C0       | 4x10^4                               | Aspergillus spp. Mucor spp. |
| M-C1       | 6x10^4                               | Aspergillus spp., Rhizopus spp. |
| M-C2       | 5x10^4                               | Aspergillus spp. Mucor spp. |
| M-C3       | 6x10^4                               | Aspergillus spp. Mucor spp. |

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml of crude oil; C1 = 200 mls of crude oil; C2 = 300 mls of crude oil; C3 = 500mls of crude oil.

Discussion

Mycorrhizal inoculation resulted in enhanced degradation of various levels of Total petroleum hydrocarbons (TPH) in the contaminated soil as observed in this study. This is in agreement with the findings of Alarcón *et al.* (2008), Khan *et al.* (2013) and Gao *et al.* (2014) where they observed an enhanced degradation of total petroleum hydrocarbons (TPH) in crude oil contaminated soils due to mycorrhizal inoculation.

The highest plant biomass (both fresh and dry) was obtained with the application of 0 ml of crude oil and this was significantly different from the other levels of contamination. The plant biomass was significantly reduced in the test crop grown in crude oil contaminated soil when compared to the control treatment and the reduction followed increase in crude oil intensity. This is in agreement with the finding of Osuagwu *et al.* (2013) where they reported reduction in bulbils yield and bulbils number in plants grown in higher levels of polluted soils compared to those in the control. Okonokhua *et al.* (2007) and Anoliefo *et al.* (2010) reported similar decrease in yield of maize and cowpea sown in oil polluted soils respectively. Merkl *et al.* (2004) working on weeds like *Centrosema brasillianum* and *P. maximum* recorded reduced biomass. Ojimba and Iyagba (2012) also reported a decrease in the output of horticultural crops planted in crude oil polluted farms compared to the unpolluted farms.

Additionally, in line with results obtained in this study, Gallegos- Martínez *et al.* (2000) also reported a reduction in plant biomass in crude oil contaminated soil.

The plant height and number of leaves of the test crop followed the same trend in this study. The highest plant height was obtained with the application of 0 ml of crude oil and this was significantly different from the other levels of contamination. This result on plant height agrees with previous findings reported by Osuagwu *et al.* (2013) where there was reduction in plant height of air potato planted in crude oil contaminated soil. Ayotamuno and Kogbara (2007) also observed and reported significant reduction in plant height of maize grown in crude oil contaminated soil. It has been extensively established in the literature that plant height as a plant growth characteristic and yield index is very important for maize. This is because the taller a plant, the higher the amount of light energy absorbed by such plant and invariably the higher the rate of photosynthesis and consequently, the higher the amount of assimilate produced by the leaves (Agbogidi *et al.*, 2007).

The number of leaves was significantly reduced in the test crop grown in crude oil contaminated soil when compared to the control treatment and the reduction in number of leaves followed increase in crude oil intensity. This supports the finding of Nkereuwem *et al.* (2010) where they reported reductions in number of leaves of amaranth crop grown in spent engine oil contaminated soil. This
reduction in number of leaves may be attributed to a host of factors including blockage of conducting tissues thereby preventing water and nutrients from entering into the plant and thus limit their ability to produce more leaves. The reductions may also be explained by the report of Amadi et al. (1996) that immediately after an oil spill, there is usually a horizontal migration of oil into soil horizons. Oily scum on soil surface would impede water and oxygen; causing some toxic elements to be more available to plants thereby causing reduction in plant growth. Therefore the general depression in growth witnessed in this study may be due to the adverse effect of crude oil.

The finding of this study is also in agreement with previous work by Parrish et al. (2005) who reported that reduction of petroleum hydrocarbon toxicity is more rapid in soils containing plants and mycorrhizal associations. The agronomic performance of costus plant grown on treatments enhanced with mycorrhiza in this study has clearly shown that mycorrhizal inoculation not only enhance the tolerance of plants to crude oil contaminated soil but can also lead to further changes in the root-zone environment thus, increasing the degradation efficiency.

The extent of root colonization by mycorrhiza decreased with increasing concentration of crude oil in the soil. The finding of this study corroborates previous report by Nwoko et al. (2014) where they observed reduced root colonization by arbuscular mycorrhiza in crude oil contaminated soil. However during the second pot trial, the level of root colonization by mycorrhiza increase with improved crude oil degradation in the contaminated soil. This may be due to the degradative ability of mycorrhiza fungi in consortium with other hydrocarbonoclastic microorganisms. This is similar to previous findings by Harrier and Watson (2004) where they reported that mycorrhizal fungi favour the activities of some soil microorganisms thus; the amount of pollutants remediated via mycorrhizal assisted remediation (MAR) is increased due to activities of these microorganisms.

**Conclusion**

Crude oil removal from contaminated soil was observed to have occurred in all the soil samples studied. However, the quantity of crude oil removal was higher in mycorrhiza inoculated samples compared to non-mycorrhiza inoculated samples. The growth and development of costus plant under stress condition may be enhanced through biological soil amendment such as mycorrhizal inoculation.

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