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

Michael E. Nkereuwem[1*], O. Fagbola[2], Iniobong E. Okon[3], Adeniyi O. Adeleye[4] and Maximilien Nzamouhe[5]

1 Department of Soil Science, Federal University Dutse, Dutse, Jigawa State, Nigeria

2 Department of Agronomy, University of Ibadan, Ibadan, Oyo State, Nigeria

3 Department of Botany, University of Uyo, Uyo, Akwa Ibom State, Nigeria

4 Department of Environmental Sciences, Federal University Dutse, Dutse, Jigawa State, Nigeria

5 Department of Soil Science and Land Resources Management, Federal University Wukari, Wukari, Taraba State, Nigeria

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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).

✉ Michael E. Nkereuwem
E-mail: michael.n@fud.edu.ng

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- Mycorrhizal application at two levels (with and without) and Crude oil contamination 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, isooctane, 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.

Table 1. Physical and chemical properties of the pre-treated soil used for the experiment.

Soil properties	Value
pH (H_2O)	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	32.22	45.78
C2	32.88	40.95	54.66	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).

Table 6: 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 2.

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	37.50
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.

Mycorrhizl	Crude oil (ml/pot)	Plant height (cm)			Number of leaves		
		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	39.56e
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
Mycorrhizl	Crude oil (ml/pot)	Fresh weight (g)			Dry weight (g)		
		4WAP	**8WAP**	**12WAP**	**4WAP**	**8WAP**	**12WAP**
M+	C0	55.92a	62.76a	116.35a	28.76a	32.91a	57.62a
M+	C1	46.13c	62.38a	89.12b	28.63a	30.64b	42.79d
M+	C2	39.01e	50.16c	66.34c	20.83c	27.30c	33.50e
M+	C3	38.95e	47.07d	63.98cd	17.93d	23.53ef	30.79f
M-	C0	53.04b	64.81a	114.30a	27.91a	31.85ab	54.22b
M-	C1	50.20d	56.36b	87.88b	22.69b	25.76d	45.62c
M-	C2	39.65e	50.78c	65.07c	20.95c	24.92de	32.62e
M-	C3	38.14e	48.81cd	61.51d	20.02c	22.40f	30.82f
	SE ()	0.84	0.84	1.03	0.44	0.51	0.53

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 n0n-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.

Mycorrhizl	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	11.11e	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
Mycorrhizl	Crude oil (ml/pot)	Plant height (cm)			Number of leaves		
		4WAP	**8WAP**	**12WAP**	**4WAP**	**8WAP**	**12WAP**
M+	C0	45.54b	55.39a	106.78a	21.17a	24.00b	46.00b
M+	C1	41.51c	55.15a	81.54b	21.84a	26.67a	38.92c
M+	C2	32.24e	44.56c	55.22de	15.05c	17.07e	23.91f
M+	C3	29.75e	39.88d	54.97de	13.20de	16.30e	22.75f
M-	C0	48.79a	53.28a	105.60a	21.64a	25.37ab	48.22a
M-	C1	36.26d	48.68b	77.69c	16.92b	20.18c	35.73d
M-	C2	32.38e	43.51cd	58.87e	13.56d	18.52d	27.69e
M-	C3	31.25e	40.95cd	57.73d	11.93e	17.10e	23.85f
	SE ()	0.85	1.28	1.07	0.52	0.49	0.52

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 mycorrhizal 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	69.30b
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 applicattion at 500 mls/pot crude oil reulted in significanthy jower mycorrhiza root colonization compared to that of 300 mls/pot crude oil.

Enteraction 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 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.

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**
Mycorrhizal			
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
Crude oil (ml/pot)			
C0	38.08	41.04	44.90
C1	34.13	37.22	41.40
C2	31.13	34.09	39.42
C3	25.49	27.48	31.19
LSD (0.05)	0.69	0.88	0.89
SE ()	0.24	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.

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	16.94g	18.73fg	22.59d
M-	C3	16.23g	18.14g	20.07f
	SE ()	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. Rhizospus spp.*
M+C3	$8x10^4$	*Aspergillus spp. Mucor spp.*
M-C0	$4x10^4$	*Aspergillus spp. Mucor spp.*
M-C1	$6x10^4$	*Aspergillus*
M-C2	$5x10^4$	*Aspergillus spp., Rhizospus 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- Martinez 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.

Conflict of interest: All authors declare no conflict of interest.

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