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## Effect of Mycorrhizal Inoculation on Growth and Nutrients Uptake of Maize Grown on Crude Oil Contaminated Soil

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### ABSTRACT

This study was carried out to evaluate the effect of mycorrhizal inoculation on the growth and nutrients uptake of maize planted in crude oil-contaminated soil. About 10 kg sterilized topsoil was contaminated with Bonny light crude oil (BLCO) at different concentrations: 0, 200, 300, and 500 ml/pot. Maize was sown at three seeds per pot and later thinned to two stands per pot after two weeks. Inoculation in treatments containing AM consisted of 20 g of *Glomus clarum*. Data on residual TPH content of the soil, plant height, number of leaves, fresh and dry weights, and nutrients uptake were collected at 4, 8, and 12 weeks after sowing (WAS). The 2 x 4 factorial experiment was laid in a completely randomized design and replicated 3 times. The results obtained indicated that AM inoculated maize had higher and significantly ( $p < 0.05$ ) nitrogen, phosphorus, potassium, calcium, plant height, number of leaves, fresh and dry weights. Significantly ( $p < 0.05$ ) lower residual TPH content was recorded with treatments inoculated with AM fungi compared to non-AM fungi inoculated treatments. AM colonization resulted in enhanced nutrients uptake thus, improving the physiological parameters of the test crop.

### INTRODUCTION

Crude oil spillage is one of the main sources of environmental pollution, especially in production areas. The environmental impact of crude oil pollution is severe on soils and vegetation on which rural livelihoods depend. Soils contaminated with crude oil are of environmental concern because such soils are not suitable for agricultural and recreational activities and are potential sources of surface and groundwater contamination (Schwab *et al.*, 1999; Adipah, 2019). Petroleum can suppress plant growth through a variety of mechanisms, including the displacement of air from soil pore spaces, an increase in oxygen demand caused by the activities of oil-decomposing microorganisms, and the creation of a hydrophobic environment, which inhibits water absorption by plant roots (Schnoor, 1997; Oyedejii *et al.*, 2012; Adipah, 2019). Soil

pollution destroys soil fertility, hamper proper plant growth, and destroys beneficial soil microbes (Uquetan *et al.*, 2017). The increasing dependence on petroleum hydrocarbons as a major energy source for both homes and industries has led to the pollution of agricultural lands due to oil spillage during exploration and processing operations.

Soil washing and flushing, solidification, stabilization, vapor extraction, etc. are some of the several physicochemical methods adopted for the rehabilitation of petroleum hydrocarbons polluted soils (Dadrasnia *et al.*, 2015; Frick *et al.*, 1999). Nevertheless, these methods are expensive, ineffective, and require continuous monitoring and control for optimum performance (Gong, 2012; Dadrasnia *et al.*, 2015; Frick *et al.*, 1999). A better strategy to overcome these drawbacks is bioremediation techniques.

Bioremediation involves the use of microorganisms (bacteria, fungi, etc.) and/or plants in the rehabilitation of organic compounds impacted soils. These techniques are economical, eco-friendly, and have shown encouraging and effective results in the treatment of soils contaminated with organic pollutants; notably petroleum hydrocarbons (Soleimani *et al.*, Graj *et al.*, 2013; Nkereuwem *et al.*, 2020a). Mycorrhizas are symbiotic relationships between plant roots and a variety of filamentous fungi that live in the soil. Arbuscular mycorrhiza (AM) fungi exhibit some exceptional traits such as vigorous growth, extensive hyphal network, adaptability to fluctuating pH and temperatures, high surface area to volume ratio, translocation of nutrients and water, production of many bioactive compounds and extracellular enzymes, and the unique capability to co-metabolize many environmental chemicals (Khan *et al.*, 2019; Singh *et al.*, 2015; Kapahi and Sachdeva, 2015; Harms *et al.*, 2011). These characteristics make AM fungi more desirable in the bioremediation of contaminated soils compared with other microorganisms (Bharath *et al.*, 2019; de Lima Souza *et al.*, 2016). Additionally, collaborative effects of AM fungi with some microorganisms in the phytoremediation process have been reported (Alcorn *et al.*, 2008; Liu and Dalpe, 2009; Teng *et al.*, 2010).

Therefore, the study seeks to: 1) determine the effect of AM fungi on growth and nutrient uptake of maize (*Zea mays* L.) planted in crude oil contaminated soil and 2) the effectiveness of AM fungi in remediation of crude oil contaminated soil as well as enhancing the survival of the test crop in crude oil-contaminated soil.

## MATERIALS AND METHODS

### Experimental site and planting materials

The study was carried out at the Department of Botany Teaching and Research Farm, University of Uyo, Uyo, Akwa Ibom State. The arbuscular mycorrhiza (AM) fungi used for the study was sourced from the Soil Microbiology Laboratory, Department of Soil Resources Management, University of Ibadan, Ibadan, Oyo State. Bonny Light Crude oil was obtained from Nigerian National Petroleum Corporation (NNPC), Port Harcourt refinery, Rivers State. Maize seed (Oba

98) was sourced from National Root Crops Research Institute (NRCRI), Umudike, Abia State.

### Soil preparation

Soil samples at depth 0-15 cm were collected, air-dried, sieved through a 2 mm sieve, sterilized, and then filled into 10 kg capacity pots.

### Planting and management

The crude oil at 200, 300, and 500 mls/pot were mixed thoroughly with the soil and allowed to stand for two weeks. Maize was sown at three seeds per pot and later thinned to two stands per pot after two weeks. Inoculation in treatments containing *Glomus clarum* consisted of 20 g of root-soil-fungal mixture blended into the central third of the soil at sowing (Carling *et al.*, 1978).

### Data collection

Plant height and the number of leaves were determined at 4, 8, and 12 weeks after sowing (WAS). The plant height (cm) was measured with the aid of a meter rule while the number of leaves was visually counted. Dry weight (g) was evaluated by oven drying the fresh biomass of the maize plant at 65°C to a constant weight. The total petroleum hydrocarbon (TPH) content of the soil was determined at 4, 8, and 12 WAS using the Soxhlet extraction method (USEPA, 2017).

### Experimental design

The experimental design was completely randomized design replicated three times. It was a factorial experiment with two factors; mycorrhizal inoculation at two levels (with and without) and crude oil contamination at four levels (0, 200, 300, and 500 mls/pot) making a total of 8 treatment combinations. The treatments were:

#### Factors:

- (a) Mycorrhizal inoculum (20 g)
  - i. *Glomus clarum* inoculation
  - ii. Without *Glomus clarum* inoculation
- (b) Crude oil (Bonny Light) contaminant
  - i. No crude oil
  - ii. 20ml/kg soil; 200 mls/pot
  - iii. 30ml/kg soil; 300 mls/pot
  - iv. 50ml/kg soil; 500 mls/pot

### Determination of the physicochemical properties of the soil sample

The particle size of the soil was determined by the hydrometer method of Bouyoucos (1951), while the soil pH was determined according to Udoh and Ogunwale (1986). The organic carbon was estimated by the modified procedure of Walkley-

Black (Nelson and Sommers, 1996). The total nitrogen and available phosphorus content of the soil were determined by the micro Kjeldahl digestion and distillation method as described by Udoh and Ogunwale (1986) and Bray P1 method (Bray and Kurtz, 1945). The exchangeable bases (K, Ca, Na and Mg) were estimated according to Jackson (1958). Ca and Mg were read off using atomic absorption spectrophotometer (AAS) while K and Na were read off using a flame photometer. Similarly, effective cation exchange capacity was determined by the summation method of Juo *et al.* (1976), while the exchangeable acidity was estimated by the method of Mclean (1982).

**Plant analysis**

Total N was determined from the digest by steam distillation with excess NaOH and boric acid indicator and then titrated with 0.01NHCl solution. P concentration was determined by the vanadomolyb date yellow colorimetric method using a spectrophotometer (Olsen *et al.*, 1954). The K was determined with the flame photometer (Jenway model) (Olsen *et al.*, 1954) while Ca and Mg were determined with Atomic absorption spectrophotometer (AAS) (Buck Scientific Atomic Spectrophotometer, Model 210/211 VGP). Nutrient accumulated in the plant was calculated thus:

$$\text{Nutrient uptake} = \% \text{ nutrient content} \times \text{dry weight of above-ground shoot biomass}$$

**The total petroleum hydrocarbon (TPH)**

The soxhlet extraction method was used to estimate the TPH content. Analytical determinations of the hydrocarbons in the soil extracts were performed by an Infrared spectrophotometer (IR). Quantification of the TPH content was carried out using the procedure described by the USEPA SW-846 series method 3540 (USEPA, 2017). After collection of the extract, it was passed through sodium sulfate and silica gel to remove water and polar constituents. An aliquot of the soil extract was then placed in the infrared spectrophotometry (IR) analyzer. The TPH value was determined by comparing it to a three-point calibration curve constructed from dilutions of a stock solution of a 2:3:3 volume ratio of chloro-benzene, isooctane, and n-hexadecane made up in perchloroethylene (PCE), according to USEPA (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 appropriate post-hoc tools.

**RESULTS AND DISCUSSION**

Table 1. Physical and chemical properties of the soil sample used in the different experimental assays

Soil properties	Value
pH (H <sub>2</sub> O)	4.9
C (g/kg)	12.4
N(g/kg)	1.0
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 <sup>+</sup>	0.82
ECEC (cmol/kg)	8.4
Particle size (g/kg)	
Sand	840
Silt	110
Clay	50
Textural class	Sandy

Table 1 above shows the physicochemical properties of the soil used for the experiment with a pH of 4.9 while the values of the major nutrient elements are as follows: Total N 1.0 g/kg, Available

P 28.70 mg/kg, exchangeable K 0.12 cmol/kg, Ca 6.0 cmol/kg and Mg 1.2 cmol/kg. The textural class of the soil is sand.

Table 2. Effect of Mycorrhizal Inoculation and Crude Oil on Plant Height and Number of Leaves of maize at different Weeks After Sowing (WAS).

Treatments	Plant height (cm)			Number of leaves		
	4WAS	8WAS	12WAS	4WAS	8WAS	12WAS
Mycorrhizal inoculation						
M <sup>+</sup>	47.14	76.07	103.91	10.28	11.69	13.47
M <sup>-</sup>	46.02	74.35	102.87	8.75	10.11	11.64
LSD (0.05)	NS	NS	NS	0.36	0.43	0.45
Crude oil (ml/pot)						
C <sub>0</sub>	69.69	128.27	160.84	11.00	12.72	14.67
C <sub>200</sub>	39.43	58.36	88.54	9.72	10.83	12.22
C <sub>300</sub>	39.26	57.52	87.29	8.83	10.17	12.00
C <sub>500</sub>	37.93	55.68	75.91	8.50	9.89	11.33
LSD (0.05)	2.01	2.88	2.38	0.50	0.61	0.64

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300 mls; C3 = 500 mls; LSD= Least significant difference, NS= Not significant.

Mycorrhizal inoculation resulted in higher plant height of maize at 4, 8, and 12 WAS however, this was not significantly higher compared with non-mycorrhizal inoculation (Table 2). Significantly higher ( $p < 0.05$ ) number of leaves of maize plant were obtained with mycorrhizal inoculation compared with non-mycorrhizal inoculation in all the weeks evaluated (Table 2)

Within all levels of crude oil application (except control), there was no significant difference in the heights of maize at 4 and 8 WAS, however at 12 WAS, maize plant height under 500 ml/pot crude oil was significantly lower (76.91 cm) compared to those of 300 and 200 mls/pot crude oil (87.29 and 88.54 cm) (Table 2). Furthermore, 200 and 300 mls/pot crude oil applications were not significantly different.

The number of leaves of the maize plant was significantly higher (10.28, 11.69, and 13.47) with

mycorrhizal inoculation compared to non-mycorrhizal inoculation (8.75, 10.11, and 11.64) in all the weeks' evaluated (Table 2).

Crude oil application at 0 ml/pot resulted in a significantly higher number of leaves of maize plant at 4, 8, and 12 WAS compared to other application levels (Table 2). Furthermore, crude oil application at 200 mls/pot at 4 and 8 WAS also resulted in a significantly higher (9.72 and 10.83) number of leaves of maize plant compared to those of 300 and 500 mls/pot (8.83 and 10.17 and 8.50 and 9.89) while 300 and 500 mls/pot were not significantly different. At 12 WAS, 200 and 300 mls/pot crude oil were not significantly different (12.22 and 12.00) but had significantly higher values compared to that of 500 mls/pot crude oil (11.33) while 300 mls/pot crude oil had a significantly higher number of leaves of maize plant compared to that of 500 mls/pot crude oil.

Table 3. Effect of mycorrhizal inoculation and crude oil on the fresh and dry weight of maize at different weeks after sowing (WAS)

Treatments	Fresh weight (g)			Dry weight		
	4WAS	8WAS	12WAS	4WAS	8WAS	12WAS
Mycorrhizal inoculation						
M <sup>+</sup>	70.30	101.77	132.50	38.06	50.44	66.35
M <sup>-</sup>	73.76	96.50	127.81	34.00	47.67	64.82
LSD (0.05)	0.96	1.07	1.38	1.05	1.13	0.98
Crude oil (ml/pot)						
C <sub>0</sub>	130.49	172.58	229.76	63.43	82.52	112.46
C <sub>200</sub>	54.83	84.70	117.26	27.82	43.83	61.21
C <sub>300</sub>	51.73	71.43	95.12	27.00	36.34	48.94
C <sub>500</sub>	51.06	67.85	78.49	25.88	33.52	39.72
LSD (0.05)	1.36	1.51	1.81	1.42	1.59	1.38

M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300 mls; C3 = 500 mls; LSD= Least significant difference, NS= Not significant

Mycorrhiza inoculated plants at 8 and 12 WAS had significantly higher fresh weight compared to non-mycorrhiza inoculated plants (Table 3) whereas at 4 WAS, non-mycorrhiza inoculated plants had significantly higher (73.76) fresh weight compared to mycorrhiza inoculated plants (70.30).

At 8 and 12 WAS, mycorrhizal inoculated plants had significantly higher dry weights (50.44 and 66.35 g) compared to non-mycorrhizal inoculated plants (Table 3) although at 4 WAS, non-mycorrhiza inoculated plant was significantly higher compared to mycorrhiza inoculated plant.

At all the weeks evaluated, significantly higher dry weights (63.43, 82.52, and 112.46 g) were

obtained with the application of 0 ml/pot crude oil compared to other levels of application (Table 3) while 500 mls/pot crude oil at 4WAS had significantly lower dry weight (25.88) compared to those of 300 and 200 mls/pot crude oil application. Crude oil applications at 200 and 300 mls/pot were not significantly different at 4 WAS. At 8 and 12 WAS, significantly higher dry weights were obtained with crude oil application at 200 mls/pot compared to those of 300 and 500 mls/pot crude oil applications while crude oil application at 500 mls/pot was also significantly lower compared to that of 300 mls/pot.

Table 4. Interactions of Mycorrhiza Inoculation with Crude Oil on Plant Height and Number of Leaves of maize at different Weeks After Sowing (WAS)

Mycorrhizal Inoculation	Crude Oil (ml/pot)	Plant height (cm)			Number of leaves		
		4WAS	8WAS	12WAS	4WAS	8WAS	12WAS
M <sup>+</sup>	C <sub>0</sub>	69.93a	130.06a	160.89a	11.89a	13.56a	15.67a
M <sup>+</sup>	C <sub>200</sub>	39.98b	58.53b	89.09b	10.33b	11.56bc	13.11bc
M <sup>+</sup>	C <sub>300</sub>	39.84b	57.53b	88.01b	9.56cd	10.89cd	12.89bc
M <sup>+</sup>	C <sub>500</sub>	38.80b	58.02b	77.70c	9.33d	10.78cd	12.22cd
M <sup>-</sup>	C <sub>0</sub>	69.44a	126.48a	160.79a	10.11bc	11.89b	13.67b
M <sup>-</sup>	C <sub>200</sub>	39.02b	58.06b	88.02b	9.11d	10.11de	1.33de
M <sup>-</sup>	C <sub>300</sub>	38.54b	57.03b	86.57b	8.11e	9.44ef	11.11e
M <sup>-</sup>	C <sub>500</sub>	37.05b	55.82b	76.11c	7.67e	9.00f	10.44e

Means with the same letter(s) in the same column are not significantly different from each other at p < 0.05 using Duncan's multiple range test, M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300mls; C3 = 500 mls

Within all levels of interaction between mycorrhizal inoculation and crude oil (except with and without mycorrhizal inoculations with 0 ml/pot

crude oil), there was no significant difference in the heights of maize plant at 4 and 8 WAS, but at 12 WAS, maize plant height under the combined

application of 500 mls/pot crude oil with non-mycorrhizal inoculation resulted in significantly lower values (76.11 cm) compared to those of with and without mycorrhizal inoculations with crude oil applications at 200 and 300 mls/pot respectively (Table 4). Furthermore, with and without mycorrhizal inoculations with 200 and 300 mls/pot crude oil were not significantly different.

Combined application of mycorrhiza with 0 ml/pot crude oil resulted in a significantly higher

(11.89, 13.56, and 15.67) number of leaves at 4, 8, and 12 WAS compared to other treatments (Table 4) while 500 mls/pot crude oil with non-mycorrhizal inoculation had significantly lower (7.67, 9.00 and 10.44) maize plant leaves compared to that of mycorrhizal inoculation with 500 mls/pot crude oil. At 8 and 12 WAS, the number of leaves of maize plants inoculated with mycorrhiza under 200 and 300 mls/pot crude oil were not significantly different.

Table 5. Interactions of Mycorrhiza Inoculation with Crude Oil on Fresh and Dry Weights of maize at different Weeks After Sowing (WAS)

Mycorrhizal inoculation	Crude oil (ml/pot)	Fresh weight (g)			Dry weight (g)		
		4WAS	8WAS	12WAS	4WAS	8WAS	12WAS
M+	C <sub>0</sub>	131.10a	173.32a	230.95a	66.90a	83.69a	111.19b
M+	C <sub>200</sub>	56.33b	86.09b	119.13b	29.38c	44.84c	62.21c
M+	C <sub>300</sub>	54.51bc	76.00c	97.55d	28.31cd	37.45d	49.76e
M+	C <sub>500</sub>	53.11c	71.68d	82.40f	27.66cde	35.76d	42.23f
M-	C <sub>0</sub>	129.89a	171.83a	228.57a	59.96b	81.35b	113.73a
M-	C <sub>200</sub>	53.34c	83.32b	115.39c	26.27de	42.82c	60.21d
M-	C <sub>300</sub>	49d	66.85e	92.68e	25.69ef	35.24d	48.12e
M-	C <sub>500</sub>	48.99d	64.01e	74.61g	24.10f	31.27e	37.20g

Means with the same letter(s) in the same column are not significantly different from each other at  $p < 0.05$  using Duncan's multiple range test, M+ = with *Glomus clarum*; M- = without *Glomus clarum*; C0 = 0 ml; C1 = 200 mls; C2 = 300mls; C3 = 500 mls

At 4, 8 and 12 WAS, significantly higher (131.10, 173.32, and 230.95 g) fresh biomass of maize was obtained from the interaction of mycorrhiza with 0 ml/pot crude oil compared to other treatments (Table 5) while significantly lower (48.99, 64.01 and 74.61 g) fresh biomass of maize plant was obtained with the combined application of 500 mls/pot crude oil with non-mycorrhizal inoculation. However, crude oil application at 500 and 300 mls/pot with non-mycorrhizal inoculation was not significantly different except at 12 WAS. In addition, mycorrhizal inoculation with 200 and 300 mls/pot crude oil and 200 mls/pot crude oil with non-mycorrhizal inoculation were not significantly different.

Mycorrhiza inoculation with 0 ml/pot crude oil resulted in significantly higher (66.90 and 83.69 g)

dry biomass of maize plant at 4 and 8 WAS compared to other treatments (Table 5), however at 12 WAS, significantly higher (113.73 g) maize dry biomass was obtained from the combined use of 0 ml/pot crude oil and without mycorrhizal inoculation. Significantly lower (24.10, 31.27, and 37.20 g) maize dry weights were obtained from the application of 500 mls/pot crude oil and without mycorrhizal inoculation compared to that of mycorrhizal inoculated with 500 mls/pot crude oil but at 4 WAS, 500 and 300 mls/pot crude oil and without mycorrhizal inoculation were not significantly different. Furthermore, at 4 WAS, mycorrhizal inoculation with 200, 300, and 500 mls/pot crude oil was also not significantly different.

Table 6. Effect of mycorrhizal inoculation and crude oil on nutrients uptake (mg/kg) in maize

Treatments	N	P	K	Ca	Mg
Mycorrhizal inoculation					
M <sup>+</sup>	3.08	0.21	0.33	2.82	0.94
M <sup>-</sup>	2.79	0.16	0.16	2.19	0.95
LSD (0.05)	0.10	0.003	0.10	0.22	NS
Crude oil (ml/pot)					
C <sub>0</sub>	3.38	0.31	0.38	3.41	1.22
C <sub>200</sub>	3.34	0.17	0.31	2.23	1.11
C <sub>300</sub>	2.68	0.15	0.17	2.22	0.76
C <sub>500</sub>	2.34	0.13	0.13	2.14	0.70
LSD (0.05)	0.15	0.004	0.14	0.31	0.05

M<sup>+</sup> = with *Glomus clarum*; M<sup>-</sup> = without *Glomus clarum*; C<sub>0</sub> = 0 ml; C<sub>1</sub> = 200 mls; C<sub>2</sub> = 300 mls; C<sub>3</sub> = 500 mls; LSD= Least significant difference, NS= Not significant

The nitrogen, phosphorus, potassium, and calcium uptakes (3.08, 0.21, 0.33, and 2.82 mg/kg) by maize plants were significantly enhanced by mycorrhiza inoculation compared to non-mycorrhiza inoculation (Table 6) while there was no significant difference in the magnesium uptake regarding mycorrhiza inoculation.

Regarding nutrients uptake by maize, significantly higher (3.38, 0.31, 0.38, 3.41, and 1.22

mg/kg) uptakes were obtained with 0 ml/pot crude oil application compared to other application levels (Table 6). Significantly lower (2.34, 0.13, 0.3, 2.14, and 0.70 mg/kg) N, P, K, Ca and Mg uptakes were obtained under 500 mls/pot crude oil application compared to that of 300 mls/pot crude oil application.

Table 7. Interaction of mycorrhizal Inoculation with Crude Oil on the nutrient's uptake (mg/kg) in maize.

Mycorrhizal Inoculation	Crude Oil (ml/pot)	N uptake	P uptake	K uptake	Ca uptake	Mg uptake
M <sup>+</sup>	C <sub>0</sub>	3.62a	0.34a	0.63a	3.82a	1.23a
M <sup>+</sup>	C <sub>200</sub>	3.27bc	0.19c	0.28b	2.64bc	1.13b
M <sup>+</sup>	C <sub>300</sub>	2.90d	0.14e	0.14b	2.31cd	0.80c
M <sup>+</sup>	C <sub>500</sub>	2.46e	0.12f	0.11b	1.97de	0.65e
M <sup>-</sup>	C <sub>0</sub>	3.41b	0.28b	0.34b	3.01b	1.21a
M <sup>-</sup>	C <sub>200</sub>	3.13c	0.17d	0.19b	2.49c	1.08b
M <sup>-</sup>	C <sub>300</sub>	2.53e	0.14e	0.14b	1.97de	0.76cd
M <sup>-</sup>	C <sub>500</sub>	2.14f	0.11g	0.12b	1.80e	0.72de

Means with the same letter(s) along the column are not significantly different from each other at (p < 0.05) using Duncan's multiple range test (DMRT)

Interaction of mycorrhiza inoculation with 0 ml/pot crude oil gave significantly higher (3.63, 0.34, 0.63, 3.82 and 1.23) N, P, K, Ca, and Mg uptakes across the weeks evaluated compared to the interaction of 0 ml/pot crude oil and without mycorrhiza inoculation (Table 7). Significantly

lower (except for K) nutrient uptakes were obtained from the combined application of 500 ml/pot crude oil and without mycorrhiza inoculation compared to that of 300 ml/pot crude oil and without mycorrhiza inoculation (N, Ca, and Mg) and mycorrhiza inoculation with 500 ml/pot crude oil (Table 7).

Table 8. Effects of mycorrhizal Inoculation and crude oil on Total Petroleum Hydrocarbon (TPH) content of soil sown with maize at different Weeks After Sowing (WAS)

Treatments	Total petroleum hydrocarbon (g/kg)		
	4WAS	8WAS	12WAS
Mycorrhizal Inoculation			
M <sup>+</sup>	59.75	51.48	46.23
M <sup>-</sup>	64.70	58.71	52.65
LSD (0.05)	0.38	0.42	0.43
Crude Oil (ml/pot)			
C <sub>0</sub>	BDL	BDL	BDL
C <sub>200</sub>	80.73	68.88	63.21
C <sub>300</sub>	83.54	73.66	65.96
C <sub>500</sub>	84.62	77.85	68.58
LSD (0.05)	0.54	0.59	0.60

M<sup>+</sup> = with *Glomus clarum*; M<sup>-</sup> = without *Glomus clarum*; C<sub>0</sub> = 0 ml; C<sub>1</sub> = 200 mls; C<sub>2</sub> = 300 mls; C<sub>3</sub> = 500 mls; LSD= Least significant difference, NS= Not significant, BDL= Below detection limit.

Mycorrhiza inoculation resulted in significantly lower (59.75, 51.48, and 46.23 g/kg) total petroleum hydrocarbon (TPH) content of the soil at 4, 8, and 12 WAS compared to non-mycorrhiza inoculation (Table 8).

The application of 200 mls/pot crude oil resulted in significantly lower (80.73, 68.88, and 63.21 g/kg) TPH content of the soil throughout the

weeks (4, 8, and 12 WAS) evaluated compared to other application levels (Table 8) while under 500 mls/pot crude oil, TPH content was significantly higher (84.62, 77.85 and 68.58 g/kg) compared to that of 300 mls/pot crude oil. However, TPH content of the soil under 0 ml/pot crude oil was below the detection limit (BDL) (Table 8).

Table 9. Interaction of Mycorrhizal Inoculation with Crude Oil on total petroleum hydrocarbon (TPH) content of soil sown with maize at different Weeks After Sowing (WAS)

Mycorrhizal inoculation	Crude oil (ml/pot)	Total petroleum hydrocarbon (g/kg)		
		4WAS	8WAS	12WAS
M <sup>+</sup>	C <sub>0</sub>	BDL	BDL	BDL
M <sup>+</sup>	C <sub>200</sub>	77.57e	64.88e	59.11f
M <sup>+</sup>	C <sub>300</sub>	80.53d	67.73d	60.86e
M <sup>+</sup>	C <sub>500</sub>	80.90d	73.32c	64.93d
M <sup>-</sup>	C <sub>0</sub>	BDL	BDL	BDL
M <sup>-</sup>	C <sub>200</sub>	83.90c	72.89c	67.31c
M <sup>-</sup>	C <sub>300</sub>	86.56b	79.59b	71.06b
M <sup>-</sup>	C <sub>500</sub>	88.33a	82.38a	72.23a

Means with the same letter(s) in the same column are not significantly different from each other at p< 0.05 using Duncan's multiple range test. BDL: Below detection limit. M<sup>+</sup> = with *Glomus clarum*; M<sup>-</sup> = without *Glomus clarum*.

The interaction of mycorrhiza with 200 mls/pot crude oil resulted in significantly lower (77.57, 64.88 and 59.11 g/kg) TPH content of the contaminated soil at 4, 8, and 12 WAS compared to other treatments (Table 9) while the interaction between 500 mls/pot crude oil and without mycorrhiza inoculation resulted in significantly

higher (88.33, 82.38 and 72.23 g/kg) TPH content of the contaminated soil at 4, 8 and 12 WAS compared to that of 300 mls/pot crude oil and without mycorrhiza inoculation.

From the results of this study, it was observed that mycorrhizal inoculation enhances the N, P, K, and Ca uptakes thus leading to enhanced plant

height, number of leaves, fresh and dry biomass weights of maize. This could be attributed to the positive effect of mycorrhiza as observed by Zhu *et al.* (2010), Ortas (2012), Crespo (2015). Mycorrhiza inoculated treatments had significantly higher nutrient uptakes compared to non-inoculated treatments. This result supports the findings of Sun *et al.* (2018), Smith and Read (2008), and Jung *et al.* (2012) that mycorrhiza fungi significantly improve plant nutrient uptake. This improvement in nutrient uptake could be attributed to the fungal mediated transport of phosphorus (P) and nitrogen (N) to plants (Smith and Read, 2008), as well as communal nutrients, being relocated from fungi to the plant, together with other related effects (Plassard and Dell, 2010) and the fungal capacity to positively influence the soil characteristics and thus supports plant development in normal as well as stressful conditions (Navarro *et al.*, 2014, Alqarawi *et al.*, 2014a&b)

The negative impact of petroleum hydrocarbons on plant growth was significantly reduced by mycorrhizal inoculation leading to improved plant growth compared with a non-inoculated plant. This implies that mycorrhiza improves the resistance of the plant to several abiotic stress factors as reported by Sun *et al.* (2018) and Birhane *et al.* (2012). There was a significant decrease in total petroleum hydrocarbons (TPH) in crude oil contaminated soil due to enhanced degradation of various levels of total petroleum hydrocarbons (TPH) in the contaminated soil as a result of mycorrhizal inoculation. This corroborates the findings of Onwuka *et al.* (2012) and Nkereuwem *et al.* (2020b) where they observed an enhanced degradation of total petroleum hydrocarbons (TPH) in crude oil-contaminated soils as a result of mycorrhizal inoculation. The result of this study is supported by the findings of Parish *et al.* (2005) that reduction in petroleum hydrocarbons toxicity is faster in soils containing plants and mycorrhizal association. It shows that mycorrhizal inoculation not only increases the resilience of plants to polluted soils by crude oil or other pollutants but also leads to changes in the rhizosphere environment thus, increasing the efficiency of degradation or breakdown.

Crude oil contamination impacted the growth indices of maize negatively as evidenced in reduced biomass (fresh and dry weight), plant height, and

number of leaves. This is in agreement with the findings of Adenipekun and Kassim (2006) that crude oil pollution is harmful to agricultural soil and this, in turn, impacts the physiological, ecological as well as anatomical development of plants cultivated in these environments. 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 corroborates the findings of Osuagwu *et al.* (2013), Okonokhua *et al.* (2007), Anoliefo *et al.* (2010), and Ojimba and Iyagba (2012), where they reported a reduction in plant height of air potato, maize, cowpea and horticultural crops planted in crude oil polluted soils compared to unpolluted soils. The reduced plant growth observed in the crude oil contaminated soil confirms the adverse effect of petroleum hydrocarbons. This reduction may be attributed to the toxic effect of the crude oil and water repellent property of hydrocarbons (Zand *et al.*, 2010). Crude oil contamination had a negative impact on the parameters evaluated and this was more prominent with an increase in crude oil contamination levels. Therefore, the general depression in growth and yield parameters as well as nutrient uptakes observed in this study is due to the detrimental effect of crude oil contamination.

## CONCLUSION

Results obtained from this study shows that mycorrhiza inoculation significantly improved the tolerance and survival of maize plant to crude oil contamination. Furthermore, growth parameters and nutrient uptake of the same plant were greatly enhanced with mycorrhizal inoculation. Additionally, the effect of crude oil contamination was more pronounced with higher levels of soil contamination. Crude oil reduction from crude oil contaminated soil was noticed in all the soil samples studied nonetheless, the quantity of removal was significantly higher in mycorrhizal inoculated treatments compared to treatments without mycorrhizal inoculation. Hence, owing to the significant TPH removal recorded in this study, the use of AM fungi in addition to planting maize or any other hydrocarbon tolerant plant in hydrocarbons impacted soil is recommended.

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