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Effect of Arbuscular Mycorrhizal (AM) Fungi on the Physiological Performance of *Phaseolus vulgaris* Grown under Crude Oil Contaminated Soil

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Abstract

An experiment was conducted to assess the influence of arbuscular mycorrhizal (AM) fungi on the performance of *Phaseolus vulgaris* under crude oil contaminated soil. *P. vulgaris* was grown on soil under 2%, 4% and 8% (v/w) crude oil contamination. The experimental units were biostimulated with 2 g NPK fertilizer pot^{-1} and were inoculated with 12 g AM inoculum pot^{-1}. Non inoculated pots served as control. The results showed that AM inoculated pots recorded higher and significantly ($P < 0.05$) different dry matter yields and chlorophyll content than non AM inoculated pots. Residual total petroleum hydrocarbon (TPH) increased as percent crude oil contamination increased. Total petroleum hydrocarbon decomposition and removal was higher on pots inoculated with AM than non inoculated pots. With AM colonization, physiological characteristics of *P. vulgaris* and TPH decomposition improved. This is evinced by the linear regression analysis between colonization and TPH ($R^2 = 0.77$).

Keywords

Arbuscular Mycorrhizae, Crude Oil Decomposition, Phytoremediation

1. Introduction

Crude oil contamination of agricultural soil often put severe stress to soil health and productivity. Record shows that crude oil spillage on arable land has been on the increase since the 20th century when global production doubled (Onosode, 2003). In the Niger delta region of Nigeria alone, about 1.8 million barrels of crude oil have been lost to the environment from 1976 to 1996 (DPR, 1999). Soil, under this condition, is much constrained to deliver the ecosystem goods and services. These constraints include soil moisture stress, low nutrient capital, and high phosphorus (P) fixation, low levels of soil organic matter, reduced soil aeration, poor soil permeability, bulk density and loss of soil biodiversity. The challenge for the next 50 years is to double food production in a more sustainable approach that will ensure public health and safety.

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Bioremediation is a low cost approach towards soil restoration. It involves the use of natural processes to contain, reduce and degrade contaminants. Various soil characteristics are essential to achieve comprehensive bioremediation of contaminated soil (Nwoko & Ogunyemi, 2010). Soil physical, chemical and biological properties are important in developing a biodegradation potential for contaminated soil (Rogers et al., 1993).

The fungi that are probably most abundant in agricultural soils are arbuscular mycorrhizal (AM) fungi (phylum: Glomeromycota). They account for 5% - 50% of the biomass of soil microbes (Olsson et al., 1999). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10 - 20 (Rillig et al., 2001). The external mycelium attains as much as 3% of root weight (Jakobsen & Rosendahl, 1990). Mycorrhizal fungi contribute to soil structure by 1) growth of external hyphae into the soil to create a skeletal structure that holds soil particles together; 2) creation by external hyphae of conditions that are conducive for the formation of micro-aggregates; 3) enmeshment of microaggregates by external hyphae and roots to form macroaggregates; and 4) directly tapping carbon resources of the plant to the soils (Miller & Jastrow, 2000). This direct access will influence the formation of soil aggregates, because soil carbon is crucial to form organic materials necessary to cement soil particles. Arbuscular mycorrhiza is an important microflora in the rhizosphere of plants and thus improve overall microbial activity in the root zone. Gao et al. (2011) observed that optimized microbiota in mycorrhizal association was responsible for PAH degradation in AM phytoremediation. Wu et al. (2011) suggested that the hyphae and extraradical mycelium of AM fungi could play important roles in the uptake and translocation of phenanthrene (PHE) and pyrene (PYR) in plants.

This present research examined the influence of arbuscular mycorrhizal fungi in the remediation of crude oil contaminated soil under African bean (*Phaseolus vulgaris*) grown pot experiment.

2. Materials and Methods

2.1. Soil Microcosm Experiment

Soil (**Table 1**) was spiked with 2%, 4% and 8% (v/wt) of crude oil (*Nigerian bonny light*) and inoculated with 12 g of mycorrhizal inoculum (16 spores·g^{-1} *Glomus mosseae*), in addition to soil resident microbes and was thoroughly mixed. Non inoculated pots were steam sterilized at 121˚C for 2 h using the autoclave and this served as control. Four Seeds of African bean (*Phaseolus vulgaris* L.) were planted per pot and thinned to two after germination. These were laid out in a simple randomized block design and replicated thrice. The pots were biostimulated by adding 2 g of NPK fertilizer pot^{-1}. The moisture content was routinely monitored and maintained at 50% water holding capacity (WHC) and average room temperature of (25˚C ± 1˚C). The experiment was left for 10 weeks in a screen house.

2.2. Analytical Methods

Soils were randomly collected from each experimental unit, homogenized, crushed and dried in the dark at room temperature under a fume hood. The soil physicochemical properties were determined using methods generally applied in soil chemistry laboratories. pH by a potentiometric method in 1:2.5 (w/v) soil water ratio. Organic carbon content was determined by wet oxidation. Total petroleum hydrocarbon (TPH) was determined using a modified EPA 8015 technique.

2.3. Mycorrhizal Colonization

To assess AM fungi colonisation, the fresh fine root sub-samples were cut into approximately 1 cm pieces,

Table 1. Initial soil characteristics.

Property	Soil	Analytical methods
Porosity (%)	51.2 ± 2.1	Brandom (1986)
Org. C (%)	0.62 ± 0.01	Wet oxidation
Total N (%)	0.41 ± 0.02	Micro kjeldhal
pH (1:2) H_2O	5.4 ± 1.2	pH meter

Org. C = organic carbon.

heated in a pressure pan at 120˚C in 10% KOH and stained using an adaptation of Phillips and Hayman (1970) protocol including a longer incubation in 2% HCl (Oliveira et al., 2001). Stained root samples were examined microscopically to assess the percentage of mycorrhizal colonisation using the grid-line intersect method (Giovannetti & Mosse, 1980).

To estimate the percentage of mycorrhizal colonization (x), intensity of infection (I) and arbuscular development (A) in the infected region of the roots were estimated in *P. vuglaris* root samples.

2.4. Plant Analysis

Dry weight of roots and shoots were determined by drying at 70˚C for 24 hrs. Chlorophyll content of plants was measured according to Harbon, (1984). The moisture content of plant tissues was determined as, an aliquot of plant sample was weighed, dried at 105˚C for 24 h, and weighed again; the difference gave the percent moisture. Data obtained were statistically analysed using Minitab software version 16.

3. Results and Discussion

The growth and development of *Phaseolus vulgaris* as influenced by crude oil contamination and AM inoculation is shown in **Table 2**. Mycorrhizal inoculation generally influenced dry matter yield. Arbuscular mycorrhizal inoculated pots recorded higher and significantly different ($P < 0.05$) yields compared to non AM inoculated pots. At 2% crude oil contamination, dry matter yield on AM was more than non AM inoculated pots. Percent crude oil contamination did not significantly affect dry matter yield on the average, low crude oil contamination (2%) had higher yields than 8%.

Chlorophyll content of *P. vulgaris* was significantly affected by the crude oil contamination and mycorrhizal inoculation. Shoot chlorophyll content decreased as percentage crude oil contamination increased. Mycorrhizal inoculation significantly enhanced chlorophyll content of *P. vulgaris* irrespective of the level of crude oil contamination (**Table 2**).

The soil chemical characteristic as influenced by the crude oil contamination is presented in **Table 3**. Soil pH was not significantly affected by all the treatments. Percent crude oil contamination did not affect percentage moisture of the residual soil. The overall effect of crude oil contamination on soil organic carbon indicated lowest (0.68%) organic carbon at 8% crude oil, 0.90% at 4% crude oil and the highest (1.16%) at 2% crude oil con-

Table 2. Effect of AM on the performance of *P. vulgaris* on various crude oil contamination level.

Parameter	Crude oil concentration					
	2%		4%		8%	
	AM	nonAM	AM	nonAM	AM	nonAM
Dry weight (g)	35.4 ± 0.87a	31.4 ± 1.61ab	33.3 ± 0.95a	27.67 ± 1.58b	34.6 ± 2.62a	16.4 ± 0.55c
Chlorophyll (µg/g)	230 ± 15.39a	170 ± 5.8b	140.6 ± 75.86c	94.07 ± 8d	121.6 ± 10.0c	72.67 ± 6.8d

AM = arbuscular mycorrhiza. Rows bearing the same letters are not significantly different.

Table 3. Residual soil chemical characteristics as influenced by AM under *P. vulgaris* grown pot experiment.

Parameter	Crude oil contamination					
	2%		4%		8%	
	AM	nonAM	AM	nonAM	AM	nonAM
pH	5.7 ± 0.1a	5.8 ± 0.05a	5.7 ± 0.05a	5.7 ± 0.05a	5.8 ± 0.11a	5.63 ± 0.2a
Org. C (%)	1.16a	0.98b	0.84ab	0.90ab	0.91ab	0.68c
TPH (mg/g)	2.23 ± 0.21e	4.1 ± 0.05c	2.96 ± 0.208d	5.66 ± 0.11b	4.6 ± 0.26c	7.13 ± 0.21a
%moisture	57.2 ± 0.72a	40.6 ± 0.5c	52.9 ± 1.2b	36.6 ± 0.8d	53.3 ± 1.05b	32.2 ± 0.52e

Org. C = Organic carbon, TPH = total petroleum hydrocarbon, AM = arbuscular mycorrhizal Rows bearing the same letters are not significantly different.

tamination (**Table 3**). Residual TPH concentration increased as percent crude oil contamination increased in the test soil. Arbuscular mycorrhizal inoculation significantly affected the decomposition of crude oil in this experiment. This is evidenced in the decrease in TPH concentration when compared to non-AM inoculated pots at different crude oil contaminations (**Table 3**). The overall assessment of impact of percent crude oil soil contamination showed no significant difference on all parameters at different levels of contamination (**Table 4**).

The arbuscular mycorrhizal colonization of *P. vulgaris* roots is shown in **Table 5**. The crude oil contamination significantly affected the level of AM colonization, development and severity of infection in this experiment. The levels of root colonization by *G. moseae* are expressed in three ways: 1) frequency of root segments (X%) reflecting the proportion of roots colonized with mycorrhizal fungi. 2) Intensity of mycorrhizal colonization in root tissues (I%). 3) The rate of arbuscular formation in root segments (A%) reflecting the potentiality of exchange with the symbiosis. From the result, the root segmentation, intensity of colonization and arbuscular formation decreased as crude oil contamination increased (**Table 5**).

The correlation matrix between AM infection on soil and plant characteristics is shown in **Table 6**. There is positive correlation ($P > 0.05$) between percentage organic carbon and arbuscular mycorrhizal development and infection in the crude oil contaminated soil. Dry matter yield and chlorophyll content of *P. vulgaris* had significant positive correlation with mycorrhizal colonization and intensity of infection. Thus, with AM colonization, the physiological characteristics of *P. vulgaris* were greatly improved. Total petroleum hydrocarbon concentra-

Table 4. Overall assessment of crude oil contamination on *P. vulgaris* and soil characteristics as influenced by arbuscular mycorrhizal inoculation.

% crude oil	pH	%moisture	TPH (mg/g)	Org. C (%)	Dry weight (g)	Chlorophyll (µg/g)
2%	5.68 ± 0.02a	49.7 ± 13.6a	2.48 ± 1.3a	0.89 ± 0.28a	31.9 ± 5.7a	206.7 ± 44.5a
4%	5.8 ± 0.17a	48.7 ± 12.4a	3.78 ± 1.6a	0.81 ± 0.06a	30.4 ± 5.1a	124.0 ± 44.3a
8%	5.83 ± 0.2a	50.2 ± 14.9a	4.3 ± 0.5a	0.76 ± 0.19a	28.7 ± 6.3a	121.4 ± 44.3a
$P < 0.05$	ns	ns	ns	Ns	ns	ns

Table 5. Percentage of AM colonization on the root of *P. vulgaris* as influenced by crude oil contamination and soil particle size.

Colonization	Crude oil		
	2%	4%	8%
X%	87a	66.3b	49.3c
I%	30.6a	26.3ab	22.6b
A%	41.0a	30.3b	20.3c

X% = Frequency of mycorrhizal root segments, I% = intensity of mycorrhizal infection, A% = rate of arbuscular development. Rows bearing the same letters are not significantly different.

Table 6. Correlation matrix of AM infection on soil and plant characteristics.

Measurement	X%	I%	A%
Org. C (%)	0.44 (0.2)	0.54 (0.13)	0.210 (0.58)
TPH (mg/g)	−0.89 (0.001^{***})	−0.92 (0.00^{***})	−0.684 (0.04^{**})
Dry weight (g)	0.734 (0.02^{**})	0.86 (0.003^{**})	0.317 (0.406)
Chlorophyll (µg/g)	0.876 (0.002^{**})	0.831 (0.006^{**})	0.80 (0.01^{**})

X = percentage mycorrhizal colonization, I% = intensity of AM infection, A% = arbuscular development.

tion showed significant negative correlation with the mycorrhizal colonization ($P = 0.001$), intensity of infection ($P = 0.0001$), arbuscular development ($P = 0.04$). At higher degree of AM infection and severity, the crude oil degradation and removal was enhanced. This is evinced by the linear regression analysis between TPH and colonization. There was strong negative relationship between the AM root colonization of *P. vulgaris* and residual TPH concentration ($R^2 = 0.77$). Soil porosity measures the total volume of pore spaces and this negatively influenced soil residual TPH concentration ($R^2 = 0.77$, $P = 0.002$).

The overall significant dry matter yield and chlorophyll content observed in mycorrhizal inoculated pots may be attributed to improved nutrient acquisition, water relations, pollutant tolerance and sequestration potentials of AM infected roots of *P. vulgaris*. One mechanism that may be involved is the oxidation of contaminant by activated oxygen species and concomitant enhancement of oxidoreductases to protect the plant from oxidative stress. Satzer *et al.*, (1999) noted enhanced levels of hydrogen peroxide in AM roots as well as enhanced levels of peroxidase activity in mycorrhizal roots and the rhizosphere which may lead to enhanced oxidation of crude oil around AM colonized root (Criquet et al., 2000). One peculiarity of crude oil polluted soil that may be overcome by AM plant is the hydrophobicity and resulting limitations in uptake of water dissolved inorganic nutrients (Leyval & Binet, 1998). There are good reasons to believe that mycorrhizal infection of roots of tropical plant species induces tolerance against abiotic and biotic stresses.

Decomposition of crude oil was significantly improved in the mycorrhizal inoculated pots as evidenced in the concentration of residual total petroleum hydrocarbon in this experiment. This important finding could be explained in the light of root physiology modification by AM that tends to increase enzyme activity level and root exudation which directly stimulates crude oil degradation. Indirect mechanisms rely on root surface properties or rhizosphere soil properties that act on crude oil availability through adsorption and co-metabolism (Jones & Leyval, 2003).

The levels of root colonization by AM were decreased with increasing concentration of crude oil in soil. Of note in this study is the behaviour of mycorrhizal activity in the contaminated soil. For example, the intensity of colonization in the root tissues and rate of arbuscular formation in root segments showed significant positive and negative correlations with dry matter yield, chlorophyll content and residual TPH, respectively. This observation reflects enhanced degradation of crude oil and symbiotic activity of AM fungi with *P. vulgaris* plant. Houngnandan *et al.*, (2000) concluded that farmers' management practices that allow a buildup of AM fungal inoculums would alleviate P-deficiency and hence increase N-fixation which will ultimately increase physiological development of the plant species. Similar interactions between AM fungi and rhizobia have been demonstrated for soybean (*Glycine max*) in low-P soils of the savanna in Nigeria (Nwoko & Sanginga, 1999; Sanginga *et al.*, 1999). AM fungal and rhizobial responses might show positive feedback. Rhizobial inoculation increased AM colonization in soybean (Sanginga *et al*., 2000) and mucuna (Houngnandan *et al*., 2001). *P. vulgaris*, a leguminous plant, may have played significant role in nitrogen fixation that further improved rhizodegradation of the crude oil contaminant.

4. Conclusion

The physiological development of *P. vulgaris* under abiotic stress may be improved through soil biological improvement strategies such as arbuscular mycorrhizal inoculation. Arbuscular mycorrhizal tends to ameliorate unfavourable conditions posed by crude oil contamination by enhanced production of oxidative enzymes and overall improvement in the soil aggregation.

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