

Response of Okra [*Abelmoschus Esculentus* (L) Moench] to Arbuscular Mycorrhizal Fungi in Crude Oil contaminated Soil

Aminanyanaba Onari Asimiea and Henry Ifeanyi Anozie

Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, Choba, Port Harcourt, Nigeria.

amina.asimiea@uniport.edu.ng

Abstract: Symbiotic organisms such as arbuscular mycorrhizal fungi (AMF) are known to play important roles in sustainable agroecosystems, improving plant performance under different environmental conditions. The influence of AMF (*Gigaspora gigantea*) on growth of Okra on crude oil contaminated soil was investigated in the Screen house of Department of Crop and Soil Science, University of Port Harcourt. The experiment was laid in a completely randomized designed (CRD) with 6 replicates. *Gigaspora gigantea* was used to inoculate 30 pots, containing 8 kg of sterilized soil contaminated with 500ml crude oil per pot at 1 weeks before planting (WAP) at the rate of 0g (Control), 10g, 12g, 14g and 16 g per pot. Results showed that AMF significantly improved the growth and nutrient uptake in inoculated pot over un-inoculated pots. Pots inoculated with AMF significantly ($P < 0.001$) increased phosphorus, nitrogen and organic carbon in post-planting analysis when compared with soil before planting (BF). The value of Total hydrocarbon content (THC) before planting (1877 Cmol/kg) was reduced to 1688 Cmol/kg after AMF inoculation. The pH value of the soil was slightly acidic with range, 5.87 – 5.92. Available Phosphorus on the AMF inoculated pots was significantly higher than that of soil before planting and Control. Total number of leaves and plant height were also higher in AMF inoculated pots when compared with the control. Pots inoculated with 16g AMF showed the optimum growth performance. This study has shown that okra grown in crude oil contaminated soils when inoculated with *Giagaspora gigantea* can overcome physiological stress caused by the contamination. Therefore, it becomes pertinent that crude oil contaminated soils should be bio-stimulated with Arbuscular mycorrhiza fungi by farmers in order to improve crop productivity.

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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 of crude oil doubled (Onosode, 2003). 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 (Nwoko, 2014).

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).

Arbuscular mycorrhizal fungi (AMF), which belong to the phylum Glomeromycota (Schubler and Walker, 2010), are ubiquitous root symbionts with low host specificity and wide geographic distribution that are associated with most species of terrestrial plants (Smith and Read, 2008). They play an important role in the balance of ecosystems by contributing to the adaptation, growth, and nutrition of plants under adverse environmental conditions (Carpio et al. 2005; Franco-Ramírez et al. 2007; Nardini et al., 2011). 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.

There are many other researches of mycorrhiza use in phytoremediation (Gerhardt et al., 2009). However, there is dearth of information on remediation done on crude oil contaminated soil using

Gigaspora gigantea. Therefore, the purpose of this study was to investigate the influence of arbuscular mycorrhizal fungi (*Gigaspora gigantea*) in the remediation of crude oil contaminated soil under Okra [*Abelmoschus Esculentus* (L) Moench] grown in pot experiment.

Materials and Methods

The experiment was conducted at the Screen House, Department of Crop and Soil Science, University of Port Harcourt. The farm is situated at latitude 6° 45 N to 7° E with an average temperature of 27°C, relative humidity of 78% and an average rainfall ranging of 2500-4000mm (Nwankwo and Ehirim, 2010).

Soils were randomly collected at depth of 0 – 30cm from fallow plot in Ibaa community, Emohua LGA, Rivers State which is situated between Latitude 4° 57'17.85" and Longitude 6°48'11.052" North of the equator on an elevation of 17.00m above sea level with a flat terrain topography. The samples were homogenized, crushed and dried in the dark room at temperature under a fume hood. The physicochemical properties of the soil were determined using standard procedures as described by Udo et al (2009) before planting. The samples were sterilized using the process of heat sterilization for 24 hours.

The soil was spiked with 500ml of Crude oil (Nigerian bonny light) and inoculated with 0g (Control), 10g, 12g, 14g and 16g of Mycorrhizal inoculum (*Gigaspora gigantea*) per pots and thoroughly mixed. Four seeds of Okra [*Abelmoschus Esculentus* (L) Moench] of Hire Variety (obtained from Agriculture Tropics, Oil Mill, Port Harcourt,

Nigeria) were planted per pot in 7litre bucket filled with 8kg of prepared soil sample and thinned to 2 seeds after germination. The experiment was laid in a completely randomized design (CRD) and with six replicates giving a total of 30 pots. The moisture content was routinely monitored and maintained at 50% water. The experiment was monitored for 8 weeks. Growth parameters like plant height, number of leaves were taken. Post-planting soil analysis was also done. The data obtained were subjected to Analysis of variance (ANOVA) and Least Significant Different was used to test for significant difference between means at 5% probability level.

Results

Soil pH

The pH value of the soil was slightly acidic with range; 5.87 – 5.92. However, pH in 10AMF (5.78) was statistically ($P < 0.05$) lower than pH values in other treatments.

Total Organic Carbon (TOC)

It was observed that TOC had the mean value of 2.85% before AMF inoculation. In post-planting analysis, there was reduction in the values of TOC, with range 2.41% (control) – 2.76% (14g AMF).

Total Nitrogen (TN)

The mean total TN value recorded in pre-planting analysis was 0.14%. However, in post planting analysis, the TN values increased with range, 0.12% (10AMF) to 0.14% (14g AMF). Control and 10g AMF values were not significantly different from each other while above 10g AMF were of significant higher values of TN (Table 1 & 2).

Table 1: Physico-chemical properties of Soil before treatment with AMF (*Gigaspora gigantea*)

pH	Sand %	Silt	Clay	TN	Avail. P Mg/kg	TOC Cmol/kg	THC
5.85	33.9	29.3	36.8	0.10	7.54	2.85	1877

TOC = total organic carbon, TN=total nitrogen, Available P=available phosphorus THC= Total Hydrocarbon Content, TOC = Total organic carbon.

Table 2. Effect of AMF inoculation on Soil Chemical Properties

Treatment	Nitrogen (%)	Avail. P (mg/kg)	pH	THC (mg/kg)	TOC
0g AMF	0.12 ^b	6.83 ^b	5.89 ^a	1696 ^a	2.41 ^b
10g AMF	0.12 ^b	13.23 ^b	5.78 ^b	1682 ^a	2.44 ^b
12g AMF	0.13 ^c	16.20 ^d	5.92 ^a	1692 ^a	2.59 ^c
14g AMF	0.14 ^a	20.22 ^c	5.85 ^a	1675 ^a	2.76 ^a
16g AMF	0.13 ^c	20.88 ^a	5.90 ^a	1675 ^a	2.72 ^a
Lsd ($p < 0.05$)*	0.04	1.006	0.15	146.2	0.07

*Lsd= Least significant difference. Means with the same alphabet in the same column are not significantly different from each other. Avail. P= Available Phosphorus, THC= Total Hydrocarbon Content, TOC = Total organic carbon.

Available Phosphorus (P)

The mean value of pre-planting analysis of P was 7.54mg/kg. It was observed that P value in post-planting analysis decreased in Control pot (6.83mg/kg) but increased in AMF inoculated pots, with highest value in 16g AMF (20.88mg/kg). However, there was significant increase ($P<0.05$) in P values in AMF

inoculated plants than Control (with exception of 10AMF values) as shown in Table 1 & 2.

Total Hydrocarbon Content (THC)

The THC value before AMF inoculation was 1877mg/kg. However the value was reduced to 1675mg/kg after AMF inoculation (post-planting analysis).

Table: 3. Effect of treatment on Plant Height (cm)

Treatment	2WAP	4WAP	6WAP
0ml AMF	2.02 ^a	1.35 ^a	0.33 ^b
10ml AMF	2.13 ^a	2.68 ^a	4.05 ^a
12ml AMF	2.30 ^a	1.98 ^a	2.02 ^a
14ml AMF	1.95 ^a	2.77 ^a	4.08 ^a
16ml AMF	1.78 ^a	3.43 ^a	4.18 ^a
Lsd ($p<0.05$)*	0.77	2.43	3.40

*Lsd= Least significant difference. Means with the same alphabet in the same column are not significantly different from each other. WAP= Week after Planting

Table: 4 Effect of Treatment on Number of Leaves

Treatment	2WAP	4WAP	6WAP
0ml AMF	0.83 ^b	0.50 ^a	0.00 ^b
10ml AMF	1.50 ^b	1.33 ^a	1.33 ^a
12ml AMF	1.50 ^b	1.00 ^a	0.67 ^a
14ml AMF	1.17 ^b	1.33 ^a	1.33 ^a
16ml AMF	1.67 ^a	1.83 ^a	1.67 ^a
Lsd ($p<0.05$)*	0.75	1.37	1.29

*Lsd= Least significant difference. Means with the same alphabet in the same column are not significantly. WAP= Week after Planting

Plant parameters

Generally, plant height and number of leaves increased more than the Control value. On 6WAP, both the plant height and number of leaves significantly increased than Control values respectively (Table 3 & 4). Maximum plant height at 4WAP (3.43cm) and 6WAP (4.18cm) was recorded when plant was inoculated with 16g AMF (Table: 3).

Discussion**Soil Parameters**

It was observed that the value of TOC in Soil before planting was reduced from 2.85 Cmol/kg to lowest value of 2.44Cmol/kg in 10AMF inoculated plant (Tables 1 & 2). This could be attributed to the ability of mycelium of AMF to degrade the crude oil due to the presence of degrading enzymes of the fungi. The values of Available Phosphorus on the inoculated pots were higher than that of soil before planting and Control (Table 1 & 2). This observation shows that phosphorus was made available due to increased

phosphorus level enhanced by the presence of mycorrhiza in the different treatments. This is in line with the findings of Smith E. S. *et al.*, (2003); Buckling and Shacker (2005) and Awotoye *et al.*, (2009) who reported that arbuscules of AMF absorb P that are beyond the reach of roots of plants. It also aids healthy plant growth that could have enhanced in-built strength to withstand physiological stress posed on the polluted soil. The Nitrogen content of the soil before and after the experiment was quite low, below the recommended critical limit of 0.15% (Solulo and Osiname, 1981; Agboola *et al.*, 1982) indicating a serious deficiency problem of Nitrogen. This could have been as a result of the absence of Nitrogen fixing bacteria that may have been killed during the heat sterilization process according to Angela J. R. *et al.*, (1983) which explains that bacteria and Ammonium nitrate was reduced due to sterilization by autoclaving and irradiation. However, the increased nitrogen content of soil could be attributed to presence of AMF which resulted in increased chlorophyll content in

leaves and increased photosynthesis, biomass production and accumulation according to the view of Olawuyi *et al.*, (2010) and Schippers, (2000) who reported the contribution of AMF in promoting the vegetative portion of plant, increasing Nitrogen availability, producing large green leaves and necessary for dropping fruits. The result also shows a marked decrease in the values of THC in all treatments when compared to the initial mean value of THC before planting (1877 Cmol/kg) as shown in Table 1 & 2. The results show that application of AMF in crude oil contaminated soil enhances the degradation of the hydrocarbons.

Plant parameters

During the experimental period, the okra plants inoculated with AMF survived under crude oil contaminated soil, grew and perform better than the control pots (without AMF inoculation). At 2 weeks after planting, the mortality rate of okra in the control pots was the highest. The results showed that plant growth parameters performed better in inoculated plants than un-inoculated okra plant irrespective of soil contamination. At 6WAP, all the leaves in Control died off due to severity of crude oil contamination. There was significant increase in plant height and number of leaves in 10g AMF inoculated plants compared to control at 6WAP (Table 3 & 4). Probably, this could be attributed to enhanced nutrient absorption in root system through arbuscular (AMF) absorptive mechanism. This observation agrees with the findings of some earlier studies by El-shaikh and Mohammed (2009) who reported an increase in agronomic growth parameters of okra when AMF was applied to soil. Generally, there was a significant increase in plant height between AMF inoculated plants and Control. The increase in plant height in AMF inoculated plants might be as a result of increased nutrient uptake. This is supported by works of George (1992) who noted that mycorrhiza increases phosphorus and micronutrient uptake and growth of their plant host resulting in significant increase in vegetative growth of okra plant compared to when there was no mycorrhizal inoculation. Maximum plant height at 4WAP (3.43cm) and 6WAP (4.18cm) was recorded when plant was inoculated with 16g AMF (Table: 3). Non-mycorrhizal inoculation (0ml AMF) resulted in lower plant height than inoculated plants as the plants may probably be deprived of phosphorus in the crude oil polluted soil. This clearly indicates that soil contamination by crude oil can impede the growth and metabolism of okra plants and the AMF inoculation could have enhanced biodegradation of various organic chemicals e.g., polycyclic aromatic hydrocarbons (PAHs) present in the crude oil thus releasing the useful mineral nutrients leading to a

boost in the shoot growth and metabolism of the growing maize plants. These observations are also in line with the results obtained by many researchers in various crops inoculated with their respective inoculum as follows: maize (Liasu *et al.*, 2002; Ayotamuno & Kogbara, 2007), tomato (Liasu, 2008) and amarathus (Olusola & Anslem, 2010). The increase in plant height in AMF inoculated plants could be attributed to increased nutrient uptake by the fungi. At 6WAI, the number of leaves per plant in all inoculated pots were significantly higher than that of Control (Table 4). This finding supports the view of Olawuyi *et al.*, (2000) who reported the contribution of Nitrogen and Phosphorus sources from AMF in promoting the vegetative portion of the plant thereby producing large green leaves nitrogen and phosphorus sources from AMF. Plants in the Control pot did not perform well in terms of growth as they rely only on the native soil nutrients which has been impeded by crude oil pollution. This is in line with studies of Dimitrow and Markow (2000) which showed that the presence of crude oil in the soil significantly decreases the available forms of phosphorus and Nitrogen to plants. These nutrients (Phosphorus, Nitrogen, Potassium and Oxygen) are essential to plant growth and development. Hence a reduction in their bioavailability which will lead to reduced plant growth and subsequent reduction in yield of the plant with low availability of food and consequently, poor economy. It was observed that 80% of the okra planted in the Control died within three weeks from the date of planting. This shows that they were susceptible to crude oil contamination which eventually led to their death. This observation is in line with the findings of Schwab and Banks (1994) which reported that the presence of hydrocarbon in the soil could be lethargic to plants. The plants leaves initially became yellow in colour (chlorosis) and eventually necrosis sets in which indicates death of the leaf cells. This confirms the findings of Quinones *et al.*, (2003), that the effect of crude oil on plants ranges from chlorosis, bleaching, spotting of leaves, necrosis, damage to the epidermal cells, yield reduction and impaired fecundity.

Conclusion

The physiological development of Okra [*Abelmoschus Esculentus* (L) Moench] under abiotic stress could be improved through soil biological improvement strategies such as arbuscular mycorrhizal inoculation. Arbuscular mycorrhizal could ameliorate unfavourable conditions posed by crude oil contamination by enhanced production of oxidative enzymes and overall improvement in the soil aggregation.

Reference

1. Agboola, A.A., G.O. Obigbesan and A.A. Fayemi, 1982. Effect of organic matter (farm yard manure), lime and phosphorus fertilizer on the yield of cowpea. *FAO Soil Bull.*, 27: 339-339. Angela J. R. A. D. Bowden, (1983) <https://www.sciencedirect.com/science/article/pii/S003807178390069X>.
2. Awotoye OO, Adewole MB, Ohiembor MO (2009) Arbuscular mycorrhiza contribution to the growth performance and heavy metal uptake of *Helianthus annuus* LINN in pot culture African Journal of Environmental Science and Technology 3: 157-163.
3. Ayotamuno, J. M., and Kogbara, R. B. (2007). Response of environmental characteristics to industrialization: A case study of the Onne Oil and Gas Free Zone, Nigeria. *Journal of Food Agriculture and Environment*, 5(1), 288-294.
4. Bucking H, Shacker-Hill Y (2005) Phosphate uptake, transport and transfer by arbuscular mycorrhizal fungus is increased by carbohydrate availability. *New Phytol* 165: 889–912.
5. Carpio, L. A., Davies, F. T., & Arnold, A. (2005). Arbuscular mycorrhizal fungi, organic and inorganic controlled release fertilizers-effect on growth and leachate of container-grown bush morning glory (*Ipomea carnea* subsp. *fistulosa*) under high production temperatures. *Journal of the American Society for Horticultural Science*, 130,131–139.
6. Dimitro, D.N. and Markow, E. (2000). Behaviour of available forms of NPK in soils polluted by oil products. *Poczwoznanie, Agrochimija Ekologia*, 35(3): 3-8.
7. Franco-Ramírez, A., Ferrera-Cerrato, R., Varela-Fregoso, L., Pérez-Moreno, J., & Alarcón, A. (2007). Arbuscular mycorrhizal fungi in chronically petroleum contaminated soils in Mexico and the effects of petroleum hydrocarbons on spore germination. *Journal of Basic Microbiology*, 47,378–383.
8. Gao Y, Li Q, Ling W, Zhu X (2011) Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene. *J Hazard Mater* 185(2–3):703–709.
9. George, E., K. Haussler, S.K. Kothari, X.L. Li and H. Marschner, 1992. Contributions of Mycorrhizal Hyphae to Nutrient and Water Uptake of Plants. In: *Mycorrhizas in Ecosystems*, Read, D.R., D.H. Lewis, A.H. Fitter and I.J. Alexander (Eds.). CAB International, Wallingford, UK, pp: 42-47.
10. Gerhardt KE, Huang XD, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. *Plant Sci* 176:20.
11. Liasu M.O., Azeez, M.A. and Atayese, M.O. (2002): Influence of AM fungal inoculation on performance of three cultivars of maize during late seasons in a savanna soil. *Science Focus*, Vol. 2, pp. 62-66.
12. Liasu, M.O. 2008. The effect of *Glomus mosseae* Nicholsen Gerdmann on establishment and growth performance of transplanted potted tomato plants in soils supplemented with composted brewery waste. *American-Eurasian J. Sust. Agric.*, 2(2): 125-134.
13. Nardini, C., Di Salvo, L., & García, D. S. I. (2011). Micorrizas arbusculares: asociaciones simbióticas e indicadores de calidad ambiental en sistemas de cultivos extensivos. *Revista Argentina de Microbiología*, 43,311–312.
14. Nwankwo C.A and Ehirim C.A. (2010) evaluation of aquifer characteristics and ground water characteristics using geo-electrode method in choba, Port Harcourt, *Journal of Scholars research library* (2) Pp 396-403.
15. Nwoko, C. O. (2014). Effect of Arbuscular Mycorrhizal (AM) Fungi on the Physiological Performance of *Phaseolus vulgaris* Grown under Crude Oil Contaminated Soil. *Journal of Geoscience and Environment Protection*, 2, 9-14. <http://dx.doi.org/10.4236/gep.2014.24002>.
16. Nwoko, C. O., & Ogunyemi, S. (2010). Effect of Palm Oil Mill Effluent (POME) on Microbial Characteristics in a Humid Tropical Soil under Laboratory Conditions. *International Journal of Environmental Science and Development*, 1, 308-314.
17. Olawuyi O.J., Odebode, A.C and Adesoye, A.I. (2010). Performance of Maize Genotypes and Arbuscular mycorrhiza in Semara District of South West Region of Doha Qatar. 4th Annual Conference of Mycological Society of Nigeria. Ekpoma, 19th – 22nd September, 2010. Book of Abstract, pp. 16.
18. Olusola, S.A. and E.E. Anslem. 2010. Bioremediation of a crude oil with *Pleurotus pulmonarius* and *Glomus mosseae* using *Amaranthus hybridus* as a test plant. *J. Bioremed. Biodegrad.* 1(3): 1-6.
19. Olusola, S.A. and E.E. Anslem. 2010. Bioremediation of a crude oil with *Pleurotus pulmonarius* and *Glomus mosseae* using *Amaranthus hybridus* as a test plant. *J. Bioremed. Biodegrad.*, 1(3): 1-6.
20. Onosode, G. O. (2003). The Vision: Niger Delta Environmental Survey. In B. A. Chokor, Ed., *Environmental Issues and Challenges of the Niger Delta* (pp. 78-98). The CIBN Press Ltd.

21. Rogers, J. A., Tedaldi, D. J., & Kavanaugh, M. C. (1993). A Screening Protocol for Bioremediation of Contaminated Soil. *Environmental Progress*, 12, 146-149. <http://dx.doi.org/10.1002/ep.670120213>
22. Schippers RR (2000). Africa Indigenous Vegetables an overview of the Cultivated Species. Natl. Res. Inst. Tech. Center Agric. Rural Cooperation, Chathan UK., pp. 89-98.
23. Schübler, A., & Walker, C. (2010). The Glomeromycota. A species list with new families and new genera. www.amf-phylogeny.com. Accessed 20 March 2015.
24. Schwab PA, Banks MK (1994) Biological mediated dissipation of polyaromatic hydrocarbons in the root zone. In: ACS symposium series 563. American Chemical Society Washinton DC pp: 132-141.
25. Quinones-Aquilar EE, Ferra-Cerrato R, Gavi RF, Fernandez L, Rodriguez VR, et al. (2003) Emergence and growth of maize in a crude oil polluted soil. *Agrociencia* 37: 585-59.
26. Smith E. S, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphorus supply to plant irrespective of growth response. *Plant Physiology* 133: 16–20.
27. Udo, E.J., T.O. Ibia, J.A. Ogunwale, A.O. Ano and I.E. Esu, 2009. Manual of Soil, Plant and Water Analysis. Sibon Books Ltd., Lagos, pp: 183.
28. Smith, S. E., & Read, D. J. (2008). Mycorrhizal symbiosis (3rd ed.). A San Diego: Academic.
29. Sobulo RA, Osiname OA (1981). Soils and fertilizer us in western Nigeria Int. Agric. Res. Train. Res. Bull. 11: 13-13.
30. Wu, F. Y., Yu, X. Z., Wu, S. C., Lin, X. G., & Wong, M. H. (2011). Phenanthrene and Pyrene Uptake by Arbuscular Mycorrhizal Maize and Their Dissipation in Soil. *Journal of Hazardous Materials*, 187, 341-347. <http://dx.doi.org/10.1016/j.jhazmat.2011.01.024>.

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