

Adaptive Capacity of two Varieties of Groundnut (*Arachis hypogaea* L.) in Crude Oil Polluted Soil

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Abstract
Arachis hypogaea L. (Groundnut) production in Niger Delta, Nigeria is limited as the crop has not been well adapted to this region. This research was carried out to determine the adaptive capability of groundnut in crude oil polluted soil. Seeds of two varieties (Java and Bold Type) were planted in soil polluted with 0.8% and 0% of crude oil. Growth parameters were measured every 10 days up to 50 days after planting. Nodules were isolated from the roots 50 days after planting and cultured in YEMA solid medium and further subcultured in PDA medium to obtain pure cultures. Result showed that they adapted to the crude oil polluted soil with Java variety having the optimum agronomic performance, root architecture and rhizobium population. Nodules formed from day 10 to day 50 after planting shows that the highest number of nodules formation occurred on day 50 for the Java variety in polluted soil (32 nodules) while the bold variety had the lowest (5 nodules). Four fungal organisms identified in the pure-cultures were *penicillium chrysogenum*, *Aspergillus niger*, *Mucor circinelloides*, *Botrydiplodia theobromae*. This research suggested that the ability of groundnut to grow in crude oil polluted soil could be due to their root architecture and ability to fix nitrogen in the soil. Further adaptation studies should be carried out covering other part of Niger Delta region.
Keywords: Groundnut, Nodule, Adaptive Capacity

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1. Introduction

Crude oil pollution is one of the rising challenges in Nigeria especially in the Niger Delta Region. This results to loss of soil fertility leading to death of plants. Studies by Adoki & Orugbai 2007 revealed that crude oil pollution inhibits growth and yield of crops. It has negative effect on the seeding germination, and growth of shoot of several plant species (Bamidele & Igiri 2011) and field grasses (Debojit *et al*. 2011). Constituent of crude oil include aromatic, aliphatic, naphthenic and oleic hydrocarbons, which has the ability to alter the physicochemical characteristics of soil and its structure. It also causes loss of soil fertility. When crude oil contamination occurs, its biological activities is hampered and it may take up to ten years to regain such activity (Wyszkowska *et al*. 2001). Example of biological activity in the soil that can be inhibited by crude oil contamination is microbial activity. Microbes such as rhizobacterial flora (rhizosphere bacteria) plays important role in the soil (Bamidele & Igiri 2011). Despite the fact that crude oil contamination plays a negative role on the growth of plant, the degree of inhibition varies from plant to plant as their genetic make-up and physiochemical properties are completely different (Bamidele, 2010, Bamidele & Igiri, 2011; Debojit *et al*. 2011). Legumes however has nitrogen fixing ability implying that they could overcome the inhibition of the contamination to a large extent. One of such legumes is the *Arachis hypogaea*, also known as the peanut, groundnut, goober, pindar or monkey, an annual legume cultivated basically for its edible seeds from the Family Fabaceae. Groundnut like other legumes has symbiotic relationship with nitrogen-fixing bacteria in their root nodules which enable the plant to fix atmospheric nitrogen in the soil (Zaid *et al*. 2019). There is however limited information on the adaptive ability of the groundnut plant in the crude oil polluted site. Hence, this study was undertaken to evaluate the adaptation of *Arachis hypogea* on crude oil polluted soil.

2. Methodology

2.1. Sample collection and Study Area

Seeds of two varieties of groundnut (Java type and Bold type) were obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. While the crude oil was obtained from Shell Petroleum Development Company Limited (SPDC)Agbada Flow Station, Igwuruta Rivers State. The study was conducted at the Center for Ecological Studies and the Pathology/Mycology laboratory of Plant Science and Biotechnology, University of Port Harcourt Nigeria.

2.2. Crude Oil Pollution Establishment of Seedlings

0.8ml of crude was added the soil and left for two weeks before seeds were established in the buckets. A total of 20 plastic buckets were filled with soil where groundnuts was previously cultivated. Five seeds each of the two varieties (bold type and java type) were sown in different holes of about 2cm depth per bucket. Each treatment consists of 0.8ml of crude oil and 0% control. Rhizobia were trapped in the root nodules of the two peanut varieties obtained from the market line for both crude oil polluted soils and the control.

2.3. Growth studies and Isolation of Rhizobia from Nodules

Agronomic data were collected for all 20 plants at 10-day interval for 50 days after sowing. Harvesting was carried out carefully so as not to destroy the nodules after 50days. The root system of each plant was washed and the fresh weight was measured after which the roots were sun dried separately in envelopes. Sun dried nodules were washed separately under tap water to completely remove soil and transferred into sterile water and kept in the refrigerator at 4°c overnight to absorb water. The nodules were then soaked for one hour in distilled water at room temperature. The rehydrated nodules were sterilized using commercial sodium hypochlorite and subjected to 95% alcohol for thirty seconds and rinsed with six changes of sterilized distilled water to remove all traces of alcohol and then in sodium hypochlorite again for 5 minutes followed by several rinses in distilled water to remove all traces of sodium hypochlorite. The nodules were then selected and crushed in a sterile petri dish using sterile forceps and then streaked on YEMA solid medium (10g mannitol, 0.2g $MgSO_4.7H_2O$; 1g yeast extract, o.5g KH_2PO_4 or K_2HPO_4, 0.1g NaCl, 1L distilled water). Petri dishes streaked with nodule preparation were incubated at room temperature for 3 days in an inverted position and observed. After incubation, separated colonies with distinct phenotypic characteristics were purified by picking up and aseptically sub culturing in potato dextrose agar and incubated for another 3 days. The streaked petri dishes were then observed and colonies counted. Isolated organism was identified with references made to the book "Food and Indoor Fungi": CBS Laboratory Manual Series.

Agronomic parameters were analyzed using Microsoft excel, 2016

3 Results and Discussion

3.1. Agronomic data

Agronomic characteristics was recorded using groundnut description of international Board of Plant Genetics Resources (IBPGR 1983). Plant height, root length, root width, plant weight, root weight, stem weight, presence of nodules and number of nodules were analyzed. Results showed the different stages of the groundnut plant growth from germination up to the formation of nodules on 50 days.

Plate 1a: Harvested varieties of groundnut at day 40 (Bold type: control (A) and polluted (B) Java type: control(C) and polluted (D)).
Plate 1b: Harvested varieties of groundnut at day 40 (Bold type: control (A)and polluted (B) Java type: control (C) and polluted (D)).

Table 1: The average agronomic data of two varieties of groundnut (Bold type and Java type) from 10-50 days after planting.

Plant Variety	No. of days	Treatment	Plant height (cm)	Root length (Cm)	Root width (cm)	Plant weight (g)	Root weight (g)	Stem weight (g)	Presence of nodules	No. of nodules
BOLD Sp.	10	Control	7.3	7.5	9.13	0.84	0.22	0.62	Absent	0
BOLD Sp.	10	Polluted	7.3	7.1	9.3	0.75	0.31	0.44	Absent	0
JAVA Sp.	10	Control	9.23	9.03	11.8	0.96	0.36	0.6	Absent	0
JAVA Sp.	10	Polluted	8.01	8.5	9.8	0.89	0.33	0.56	Absent	0
BOLD Sp.	20	Control	11.6	11.35	16.5	1.37	0.63	0.74	Present	9
BOLD Sp.	20	Polluted	11.2	11.31	16.65	1.33	0.61	0.72	Present	6
JAVA Sp.	20	Control	12.6	9.8	17.2	1.66	0.71	0.95	Present	7
JAVA Sp.	20	Polluted	11.8	10.5	16.85	1.53	0.74	0.79	Present	5
BOLD Sp.	30	Control	14.5	12.5	18.5	4.63	0.97	3.66	Present	19
BOLD Sp.	30	Polluted	14.2	13.6	18.9	4.21	0.93	3.28	Present	17
JAVA Sp.	30	Control	15.6	12.9	17.6	4.82	0.95	3.87	Present	16
JAVA Sp.	30	Polluted	15.1	12.8	15.3	3.99	0.89	3.1	Present	19
BOLD Sp.	40	Control	19.1	18.9	22.16	7.52	1.94	5.58	Present	21
BOLD Sp.	40	Polluted	15.2	15.8	18.26	6.95	1.57	5.36	Present	32
JAVA Sp.	40	Control	16.5	17.5	16.7	7.83	1.86	5.97	Present	29
JAVA Sp.	40	Polluted	18.1	20.06	21.3	7.18	1.85	5.33	Present	17
BOLD Sp.	50	Control	19.2	21.9	23	11.07	3.07	7.97	Present	25
BOLD Sp.	50	Polluted	18.5	19.5	21.8	9.54	2.88	6.66	Present	29
JAVA Sp.	50	Control	19.9	22.3	20.6	11.51	1.56	9.95	Present	28
JAVA Sp.	50	Polluted	17.8	20.1	21.5	9.91	2.35	7.56	Present	25

Plant height

From the result it was observed that the Java variety of groundnut in control soil had the highest height (19.9cm) at day 50 while Bold variety had the lowest height (7.3 cm) for both control and polluted at day 10. Generally, both varieties of groundnut in unpolluted soil had the highest heights from day 10 to day 50 except in the Bold type that were found to be of the same height on day 10.

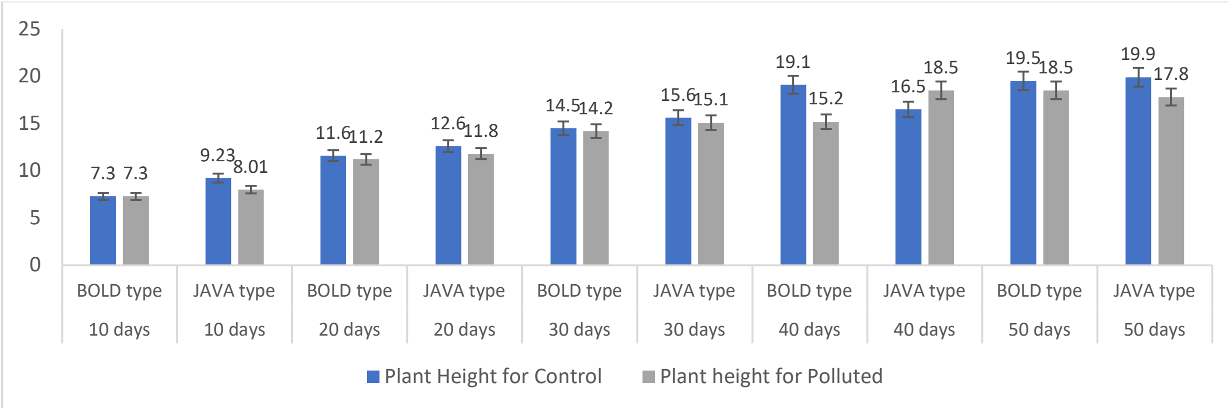

Figure1: Plant height (cm) for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil.

Root length

The Java type in unpolluted soil had the highest root length of 22.3cm while the Bold type in the polluted soil had the lowest root width of 7.1cm.

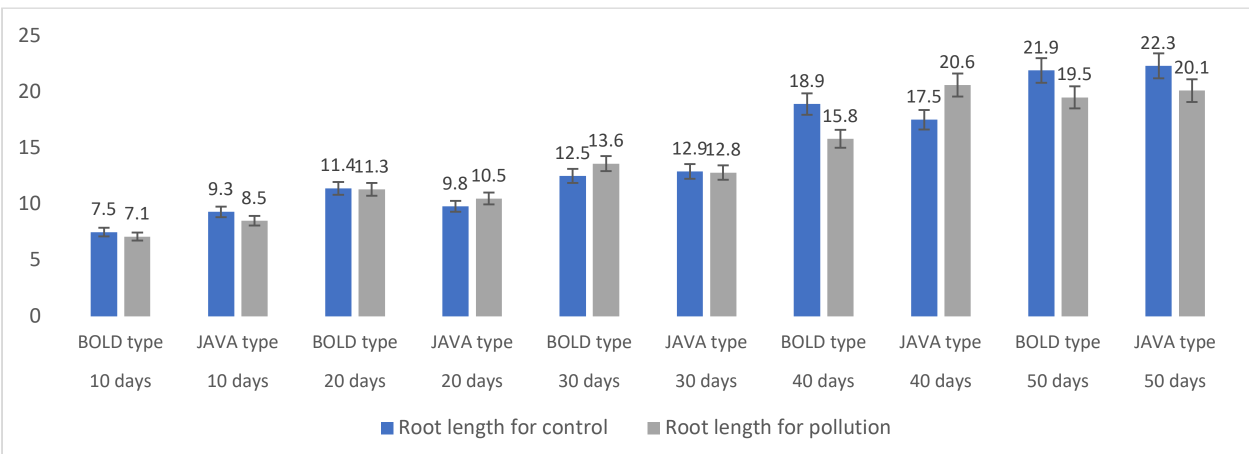

Figure 2: Root length (cm) for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil.

Plant weight

The highest and lowest weight occurred on the 50th and 10th day. The Java type in unpolluted soil had the highest plant weight of 11.51g while the Bold type in the polluted soil had the lowest weight of 0.75g.

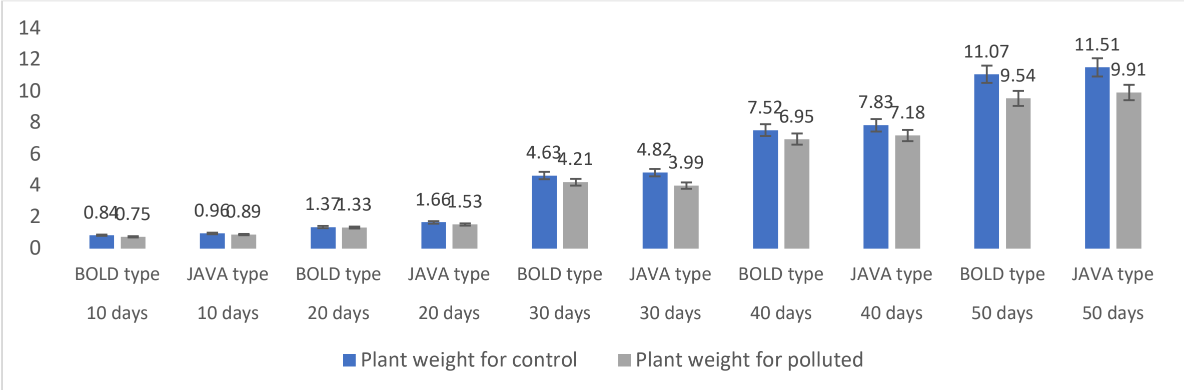

Figure 3: Plant weight (g) for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil.

Root width

The bold variety of groundnut in polluted soil had the highest root width of 23cm while the bold variety planted on the unpolluted soil had the lowest root width of 9.13cm

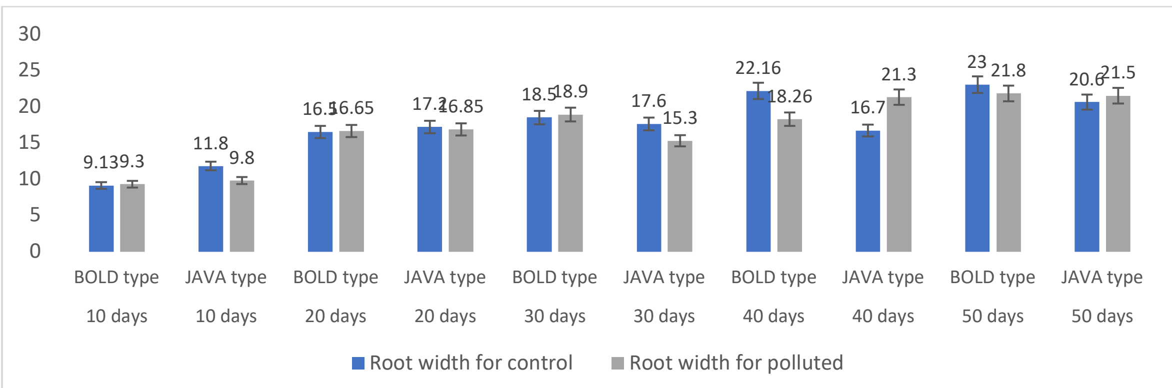

Figure 4: Root width (cm) for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil

Root weight

The highest and lowest weight occurred on the 50th and 10th day. The Bold type in the unpolluted soil had the highest root weight of 3.07g. While the Bold type in the unpolluted soil also had the lowest weight of 0.22g.

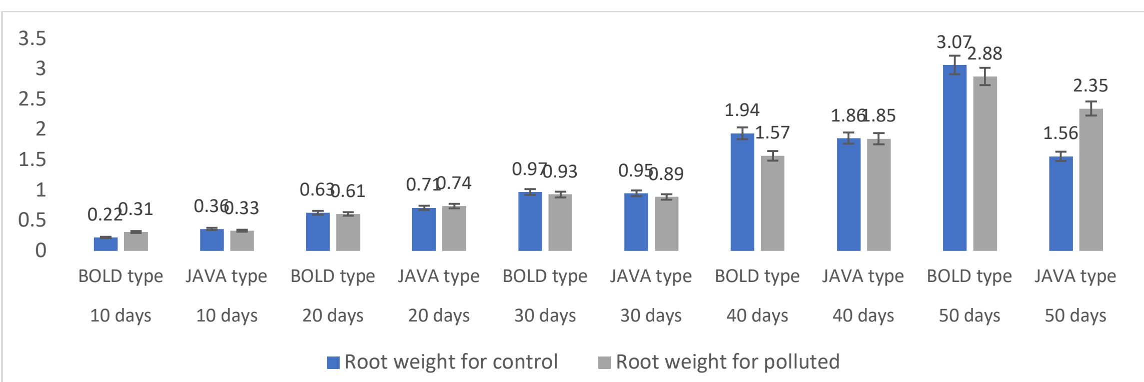

Figure 5: Root weight (g) for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil.

Stem weight

From the result it was observed that the varieties of the groundnut plants had a stem weight ranging from 0.44g to 9.95g. The highest and lowest weight occurred on the 50th and 10th day respectively. The Java type in the unpolluted soil had the highest stem weight of 9.95g. While the Bold type in the polluted soil had the lowest weight of 0.44g.

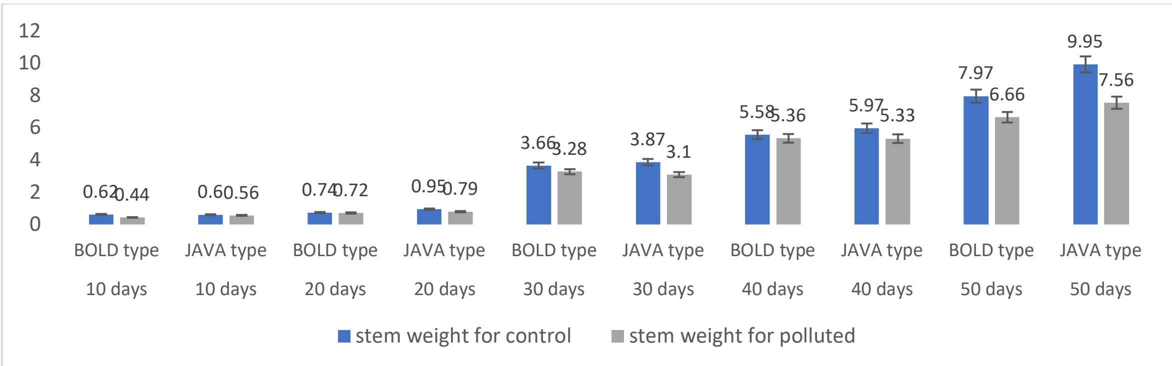

Figure 6: Stem weight (g) for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil.

Number of nodules

The highest and lowest numbers of nodule formation occurred on the 40th and 20th day respectively. Interestingly the bold type in the polluted soil had the highest numbers of nodule formation (32) while the Java type in the polluted soil also had the lowest numbers of nodule formed (5). Nodules were not formed on day 10.

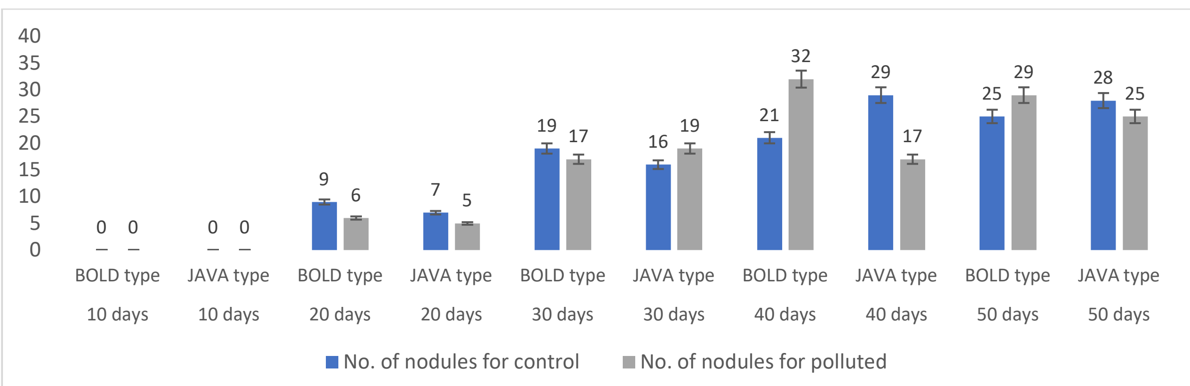

Figure 7: Number of Nodules for the two varieties of groundnut after 10 to 50 days in a crude oil polluted and unpolluted soil.

Root architecture

There were gradual and similar changes in the root architecture and morphology for the roots in both the unpolluted and polluted soils from the 10 days to the 30 days period in both cowpea varieties. At the 10th day, there were some adventitious roots with shallow basal roots, while the 30th day old roots had more adventitious roots that were smaller in diameter, had shallower basal roots with more dispersed laterals. There was greater root biomass with longer and denser root hairs.

Plate 2a: The root of the two varieties of groundnut

Isolation of nodule population

Colonies showing different morphological characteristics on the plates. Fungi identified were *Penicillium chrysogenum, Botryodiplodia theobromae, Mucor circinelloides, Aspergillus flavus, Aspergillus spp, Neurospora spp and Aspergillus niger* (Plate 3.a-3f).

Plate 3a: Growth of Arbuscular mycorrhizas extracted from crushed nodules cultured on Yema Medium
Plate 3b: Sub cultured isolates in PDA medium

Plate 3c: P*enicillium chrysogenum* in PDA media Plate 3d: *Aspergillus niger in* PDA media

Plate 3e: ***Mucor circinelloides*** **In PDA media**

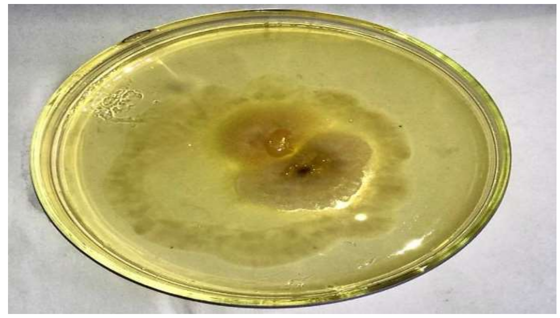

Plate 3f: ***Botryodiplodia theobromae*** **in PDA media.**

Result from this study shows that the variety of groundnut with the highest plant height after 50 days of planting was the control for Java type with a height of 19.9cm. Generally, both varieties of groundnut in unpolluted soil had the highest heights from day 10 to day 50 except in the bold type that were found to be of the same height on day 10. From the agronomic features, it was observed that there was a gradual increase in the plant height, shoot and root length, root width, plant and the number of nodules according to the number of days after germination except for nodules formation where the numbers of nodules formed (32) on day 40 for the bold type in the polluted soil was the highest. It was also discovered that the unpolluted soil produced the highest root length (22.3cm), highest plant weight (11.51g), and the highest root weight (3.07) and the highest stem weight (9.95). However, the study showed that there was no significant difference in the agronomic characters of both studied species compared to both polluted and control soil. This shows that the presence of AMF is therefore considered to be beneficial for groundnut due to their effectiveness even in areas with innately little available soil nutrient and can help in assembling nutrients bound to biological materials and particles of soil for the growth of plant. The results from the root architecture indicated that there were gradual and similar changes in the root architecture and morphology for the roots in both the unpolluted and polluted soils from the 10 days to the 30 days period. According to Lynch and Brown (2008), groundnut plants adapting to soils with low nitrogen and phosphorus usually develop more adventitious roots that were smaller. In addition to other arbuscular mycorrhizas isolated from the nodules of the two varieties of *Arachis hypogea*, a research by Ephrem (2015) analogous to this study in Ethiopia revealed that *A. niger and A. flavus* which were isolated from all samples; 29-60 of A. *niger* occurred in 29–60% and *A.flavus* in 4–52% of kernels. *Rhizopus* spp., *Penicillium* spp. and with moderately *Sclerotium bataticola* were copious in samples. The plant root is in symbiotic relationship with Arbuscular mycorrhizal fungus (AMF). The AMF has an additional radical hypha that acts as extension of roots that successful transport phosphate from the soil to the plants for their utilization. This conceivable role of AMF in terms of their involvement in phosphate nutrition has currently gained much importance (Ramos-Zapata *et al*. 2009). In the same vein Der Heijden *et al* (2011) highlighted importance of mycorrhizal fungus on crop production viz it boosts nutrient acquisition, increases establishment of seedling, fight against drought and pollution. Most importantly, if serves as mediator in Nitrogen fixation, and improves the structure of soil (Zaid and Wani, 2019). they have the potentiality of producing soil nutrient in nutrient deficient area hence act as biofertilizer (Karandashov and Bucher 2005). They symbiotic association with the root of legume also enhances the growth of these legumes (Der Heijden *et al*. 2015). These characteristics of the AMF tells a lot about the adaptive capability of the groundnut varieties study.

4. Conclusion

The results obtained from this study showed that the java variety in the control treatment on the 50th day showed the highest plant height, root width, plant weight and stem weight recorded while the bold variety in the polluted soil recorded the lowest in the plant height, root width, plant weight and stem weight. The highest number of nodules formation occurred on the 50th interestingly for the java variety in polluted soil (32 nodules) while the bold variety in polluted soil had the lowest (5 nodules). The results on the isolation of rhizobia from the nodules shows the presence of four fungal organisms in the pure-cultures namely; *penicillium chrysogenum*, *Aspergillus niger*, *Mucor circinelloides*, *Botrydiplodia theobromae*. It was concluded that the java variety adapted best in crude oil polluted soil for the growth parameters, root architecture and rhizobium population monitored and therefore *Arachis hypogaea* L. has the ability to grow in crude oil polluted soil because of its root architecture and ability to fix atmospheric nitrogen in nutrient inherent soil. Further study is recommended to be carried out to evaluate the adaptation of groundnut various in the agro-ecological zone of the Niger Delta and also by using farmers preferred varieties.

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