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Response of African Yam Bean to Charcoal and Calcium Chloride Treatment in a Crude Oil Polluted Soil

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ABSTRACT: The Response of African Yam Bean to charcoal and calcium chloride treatment in a crude oil polluted soil was carried out. 10kg of soil was polluted with 600ml of crude oil and was remediated with 500g of charcoal; the germinated seeds were pre-treated with 25g/l of Calcium chloride for 15mins before it was sown. The different treatments were: Control, 6% pollution, 6% pollution and charcoal, Charcoal only, Pre-treatment with calcium chloride, Pre-treatment with calcium chloride and 6% pollution, Pre-treatment with calcium chloride and 6% pollution and charcoal, Pre-treatment with calcium chloride and charcoal. Results showed that crude oil pollution impacted negatively on the parameters studied, the addition of materials (Charcoal and Calcium chloride) to the crude oil polluted soil improved the growth of African yam bean. Calcium chloride pre- treatment inhibited the emergence of AYB in a polluted soil. There was a significant difference ($P>0.05$) in the plant height, leave area, number of leaves, carbohydrate content, stem girth and root length, while treatments had no significant difference in the chlorophyll content and number of roots. The combined treatment ($CaCl_2$ + Charcoal + pollution) did not show any significant impact on the growth and biochemical parameters when compared to the single treatments therefore; Charcoal and Calcium chloride are very good biostimulants but are more effective when they are applied separately.

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Many communities located in the Niger Delta part of Nigeria are faced with the prevalence of crude oil pollution which has led to loss of soil fertility and the sustainability of agricultural lands, loss of jobs for occupants and indigene of affected communities, and death of plant and animals. (Agbogidi, 2010). With consequent Crude oil pollution on agricultural lands many heavy metals have been accumulated in the soil, these metals are absorbed into the plants which can lead to yield reduction and death of plants. Crude oil influences the chemical and physical properties of soil as well as plant growth parameters (Wang *et al.*, 2013). Ihimikaiye and Tanee (2015); reported that a high concentration of spent carbide waste impaired the germination growth, development and yield of African Yam Bean and Cowpea. Bioremediation of hydrocarbon products is the magic potion that can be employed to restore and reclaim the environment in order for living organisms to thrive (Adetitun, *et al.*, 2016). Bioremediation is cheaper, saves time and is environmental friendly. According to Ofoegbu *et al.*, (2014), the nature and concentration of contaminants, edaphic properties, moisture, temperature and oxygen are some environmental factors that can influence bioremediation. In recent times, the use of Organic and Inorganic fertilizers to biostimulate crude oil degraders have proven to be an effective tool of bioremediation (Agarry, 2018).

The biostimulants used in this research are Charcoal and Calcium Chloride ($CaCl_2$). Charcoal is an organic material made from pyrolysis of organic materials. It aids in the formulation of many soil nutrients and can improve soil structure, plant growth and the increase of soil microbial population (Mensah and Ekeke, 2016). Charcoal is capable of absorbing organic and inorganic contamination in the soil due to its high cation exchange capacity (Ndor *et al.*, 2016). Charcoal improves photosynthesis (Viger *et al.*, 2013); it increases the formation of root nodules in legumes and invigorates the production of plant growth hormones (Lehmann, 2009). Calcium chloride ($CaCl_2$) is a salt,

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and an ionic form of chlorine and calcium compound. According to Xu *et al*., (2013) Calcium chloride improves plants tolerance to heavy metal stress and it is also a source of some macro and micro nutrients; they reported that calcium chloride pretreatment increased the dry biomass, above ground and below ground fresh biomass of *Zoysia japonica*. African yam bean (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich) is an herbaceous plant, it belongs to the Fabaceae family of leguminous plants, it is commonly known as African Yam Bean (AYB).

It is characterized by alternately arranged leaves, long glabrous pods, with twinning stems, its seeds vary in colour. It is predominant in tropical Africa. African yam bean is a less known legume but with a great potential for food security in Africa. African yam bean contains high amounts of protein which makes it suitable animal feed, its root nodules aids in nitrogen fixation thereby improving soil fertility (Adewale *et al*., 2013).

Therefore, the research work explores the effect of crude oil pollution on the growth of African Yam Bean and how organic additives such as charcoal and inorganic additive such as Calcium chloride can be used to improve the growth and development of plants in a polluted soil.

MATERIALS AND METHOD

Experimental Site Location: The research was conducted at University of Port Harcourt, Port Harcourt, Rivers State; Lat. 4◦ 54' 31" N and Long.6◦ 54' 38" E. The temperature range from 23°c to 35°c and total rainfall range of 2000-3000mm per year, from May 2017 to May, 2019; the soil within this area ranges from well drained to medium well drained (Opara-Nnadi *et al*.,1987). This experiment was conducted between July and September.

*Sources of Planting and Pollutant Materials***:** Seeds of *Sphenostylis stenocarpa* (African Yam Bean), were obtained from Mile one market, Port Harcourt. The charcoal was purchased from the Choba Market while Chemical retail outlet at Port Harcourt supplied the calcium chloride. The crude oil was gotten from Nigeria National Petroleum Corporation (NNPC)

Experimental Soil: Loamy- sandy soil was collected from the faculty of Agriculture teaching farm, University of Port Harcourt, within a depth of 0-15cm. the soil was then filled into 24 polybags. 10kg of soil was placed in each bag, the bags were punctured to avoid water logging and to increase soil aeration. with a radius of 11cm and a height of 16cm leaving a space of 5cm from the top end of the bag to allow for addition of pollutant and amendment.

Application of Treatments: 600ml of crude oil was added to each bag of soil and the unpolluted soil served as the control, the polluted soil was allowed for 7days to homogenize before the addition of the amendment material. 500g of charcoal was weighed using the sensitive weighing balance and added to the soil, the charcoal was mixed thoroughly with the soil using a garden trowel. The mixed soil was allowed to homogenize for another 7days before planting commenced. The seeds of *Sphenostylis stenocarpa* were germinated in a Petri dish and after 5days it was pre-treated with Calcium chloride. Seeds were soaked in 25g/l of calcium chloride for 15miuntes before planting.

Experimental Design: This study was arranged in a randomized complete block design (RCBD) having eight Treatments and three replicates and the treatments are as follows; Control, 6% pollution, 6% pollution and charcoal, Charcoal only, Pre-treatment with calcium chloride, Pre-treatment with calcium chloride and 6% pollution, Pre-treatment with calcium chloride and 6% pollution and charcoal, Pre-treatment with calcium chloride and charcoal.

Growth and Biochemical Analysis: The following are growth and biochemical parameters analysed in this study: plant height, leaf area, and number of leaves, stem girth, root length, number of root, chlorophyll and carbohydrate content. Data was collected at a 2 weeks interstice for 12 weeks.

Plant Height: The plant's height was estimated with the meter rule in centimetres, from base of the vine to its tip.
Number of Leaves: The number of leaves was estimated by manual counting of the leaves.

Leaf Area: The plant leaf area was determined by using the non-destructive method, following the method of Agbogidi and Ofuoko (2005). Measurement of the leaf multiplied the correctional factor of the width and length for cowpea 0.75. Where A= 0.75× K; A= leaf area of AYB, K= width and length of the leaf measured.

Stem girth: The stem girth was estimated by placing a venire calliper at 3cm above soil level of the plant stem, and then adjusting and reading the scale. The venire calliper was calibrated in centimetres. The number of root was obtained by carefully loosening the potted plants and counting the roots. The root length was determined by measuring the length of the root with a meter rule in centimetres, after the plants were harvested.

Leaf chlorophyll: leaf chlorophyll content was extracted from 1.0g of fresh leaf sample added in 10ml of acetone; the solution was kept at room temperature for 2 days. The colour of the solution change to green, the solution was read at an optical density at 660nm and 643.

Leaf carbohydrate: Leaf carbohydrate content was measured using the Anthrone method.1.0g of dry leaf sample and digested in 62% Perchloric acid and the glucose content was determined colorimetrically.

Statistical Analysis: One-way analysis of variance (ANOVA) was applied to determine the significant difference within treatment over 12weeks (Weeks after planting) at 5% significant level. Also, multiple comparisons are applied to determine significant difference between paired treatments. Descriptive statistics such as mean and standard error was used. These analyses were aided using SAS version 9.1 statistical package.

RESULTS AND DISSCUSIONS

The heights attained by AYB plants grown in different treatments are presented in Figure 1. Results of the effect of $CaCl_2$ pre-treatment and charcoal on the plant height of African Yam Bean (AYB) grown in a crude oil polluted soil are shown in Figure 1. Results obtained indicated that AYB sown on crude oil and $CaCl_2$ pretreatment suffered a lapse in terms of emergence. Plant height of AYB was significantly different ($P > 0.05$) from 2WAP to 12WAP. Highest significant plant height was recorded in charcoal treatment, followed by the control, while treatment crude oil only recorded the lowest plant height. In figure 2, There was a decrease in the number of leaves of AYB sown in crude oil only, crude oil + charcoal, and $CaCl_2$ + crude oil + charcoal also, treatments of Charcoal only and charcoal + $CaCl_2$ were most effective in increasing the number of leaves. There were significant ($P > 0.05$) reductions in plant leaf area of AYB in crude oil polluted soil (figure 3. 3). There was a decrease in the leaf at 12WAP except for treatments crude oil + charcoal, $CaCl_2$ only, and $CaCl_2$+ charcoal.

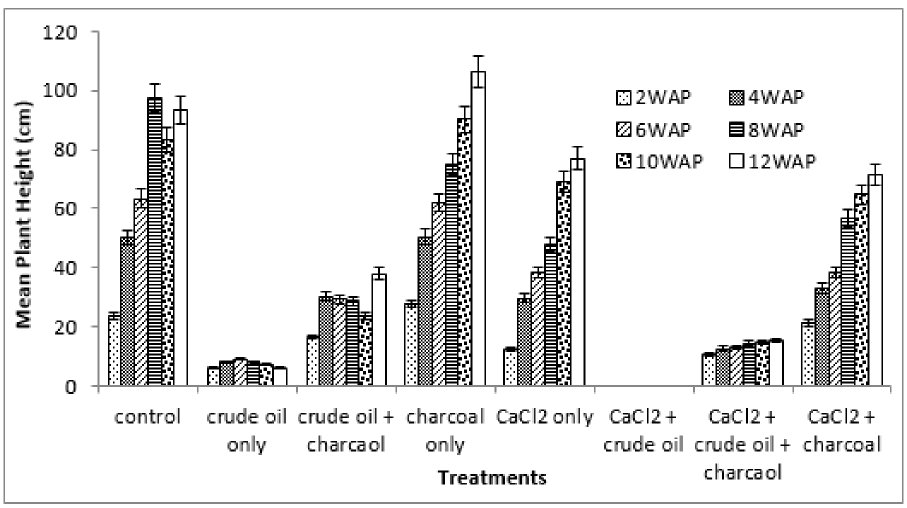

Fig 1: Mean plant height of AYB

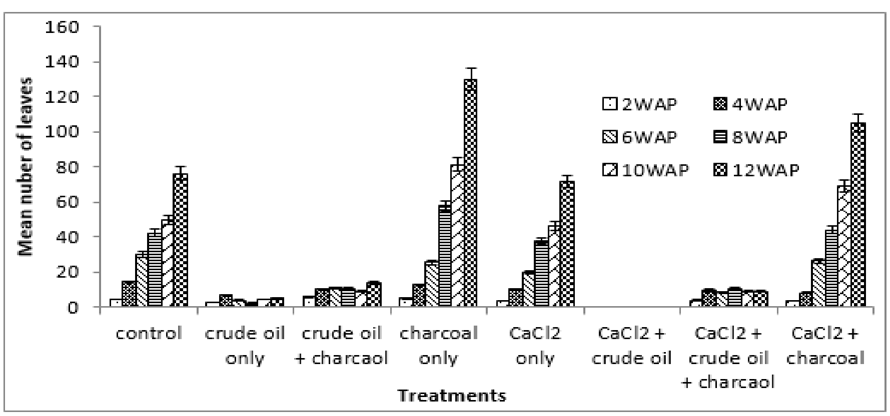

Fig 2: Mean number of leaves of AYB

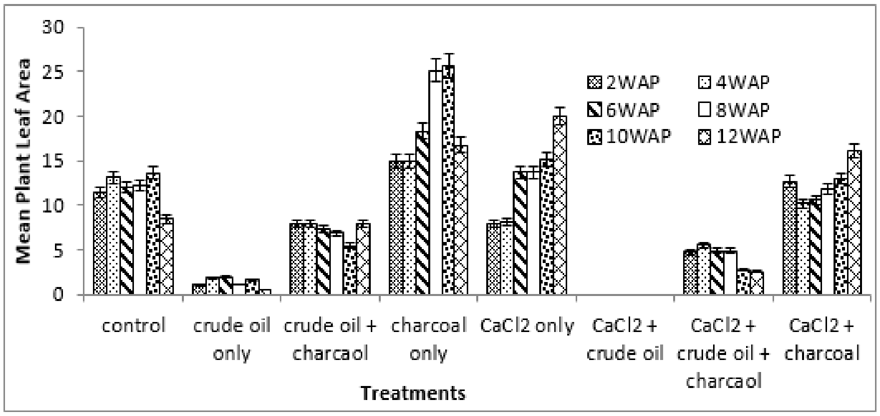

Fig 3: Plant leaf area of AYB

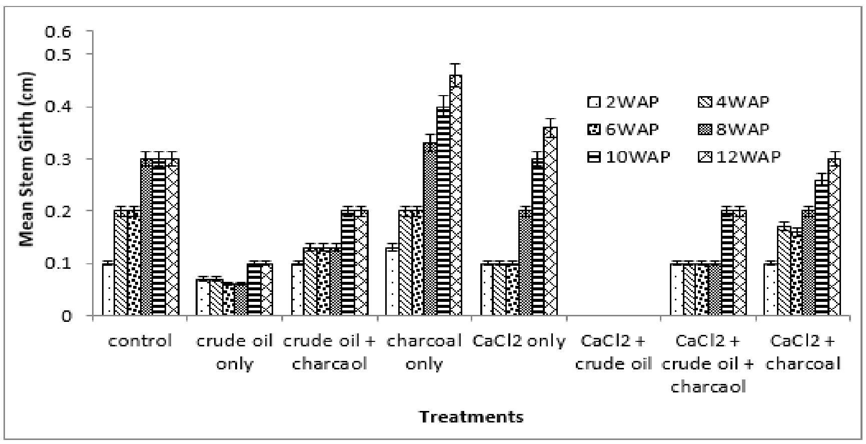

Fig 4: stem grith of AYB

The stem girth of AYB is shown in figure 4. At the initial stage, treatments of crude oil only, $CaCl_2$ only, and $CaCl_2$ + crude oil+ charcoal recorded similar girth measurements. Whereas, treatments of control, charcoal only, and $CaCl_2$ + charcoal; increased differently. Nevertheless, there was a significant difference ($P > 0.05$) in the girth measurement after each week. Table 1 shows no significant ($P<0.05$) difference in the leaf chlorophyll content in the various remediation treatment. The highest leaf carbohydrate content was recorded in the treatment with charcoal only; there was a significant difference in the carbohydrate content among treatments. In Table 2, the effect of charcoal and calcium chloride treatment on root length and root number is shown. There was no significant difference ($P<0.05$) in the number of roots. The highest root length was recorded in the control treatment and there was significant difference among the various treatments.

Table 1: Effects of charcoal and calcium chloride on the chlorophyll and carbohydrate content of *Sphenostylis stenocarpa* grown on crude oil polluted soil.

Treatment	Chlorophyll Content	Carbohydrate content
Control	7.72±4.08[a]	7.93±0.99[a]
Crude oil	5.01±1.80[a]	5.42±1.76[a]
Charcoal + crude oil	6.07±4.57[a]	8.98±0.93[a]
Charcoal only	2.89±1.03[a]	10.91±0.5[a]
$CaCl_2$	9.75±6.68[a]	7.96±1.23[a]
$CaCl_{2\,+}$ crude oil	0.00±0.00[a]	0.00±0.00[a]
$CaCl_{2\,+}$ crude oil$_+$ Charcoal	2.69±1.65[a]	7.45±1.16[a]
$CaCl_{2\,+}$ charcoal	4.96±1.33[a]	6.51±3.53[a]
ANOVA (p-value)	0.581	0.009*
Significance at (P≤ 0.05)	NS	S

S= significant; NS= not significant. Mean ± standard error mean represents 3 replicates.

Table 2: Effects of charcoal and calcium chloride on the root height and number of roots of *Sphenostylis stenocarpa* grown on crude oil polluted soil.

Treatment	Root Length	Root Number
Control	37.66±10.41[a]	8.0±0.57[abc]
Crude oil	9.00±1.52[a]	4.0±0.57[a]
Charcoal + crude oil	8.66±2.02[abc]	3.0±1.0[bc]
Charcoal only	24.0±1.0[ab]	9.0±2.08[ab]
$CaCl_2$	24.33±9.24[ab]	12.66±6.69[a]
$CaCl_2$ + crude oil	0.00±0.00[a]	0.00±0.00[a]
$CaCl_2$ + crude oil + Charcoal	17.66±1.45[bc]	7.66±0.66[bc]
$CaCl_2$ + charcoal	24.33±8.17[ab]	6.0±1.15[abc]
ANOVA (p-value)	0.008*	0.073
Significance at ($P \leq 0.05$)	S	NS

S= significant; NS= not significant. Mean ± standard error mean represents 3 replicates

The AYB plant grown in crude oil polluted soil suffered from stunted growth when compared to the control. This is due to high degree of toxicity causing damage to shoot tissues which results in difficulty in the transportation and absorption of nutrients (Eremrena and Akonye, 2013). Soil with treatment $CaCl_2$ + crude oil did not allow for seed emergence (Fig. 1). In Ekpo and Ebeago (2009), they reported that crude oil is responsible for poor emergence and seed mortality of many legumes sown in polluted soil; this is because of the sorption of oil in the seed embryo. The addition of charcoal to crude oil polluted soil improved the plant height of AYB (Fig.1). Charcoal is associated with the overall improvement of soil pH, soil water holding capacity and a high cation exchange capacity which fosters nutrient availability and increase plant growth (Paz- Ferreiro *et al*., 2013). $CaCl_2$ pre-treatment increased the plant height of AYB, this is in agreement with Offor *et al*., (2016), wherein it was indicated that the pre-treatment of cassava variety TMS 3072 with $CaCl_2$ and water significantly increased the plant height. Workers have shown that $CaCl_2$ is a good physiological treatment for plants undergoing abiotic stress (Xu *et al*., 2013). The stem girth was equally affected by various remediation treatments (Fig.4). Crude oil severely reduced the stem girth of AYB when compared to the control. The addition of charcoal and charcoal + $CaCl_2$ increased the lateral expansion of the plant. Earlier, Abukari (2014) stated that the addition of charcoal increased the stem girth of maize; this may be attributed to the increased water holding capacity of the soil. Fagbenro *et al*., (2013) also indicated that the stem girth of maize was significantly improved when charcoal was added to the soil. The number of leaves of AYB sown in crude oil only was drastically reduced, and there was no significant difference between treatment crude oil only and crude oil + charcoal (Fig. 2). Crude oil disrupts the synthesis of important materials for the formation leaves; it can also lead to wilting and leaf necrosis (Ogbuehi and Ezeibekwe, 2009). The treatment charcoal only had the highest mean number of leaves, this is in agreement with Mensah and Ekeke (2016) and Sokchea and Preston (2011). Crude oil pollution affected leaf area of AYB; it is responsible for deficient photosynthesis, respiration and stomata dysfunction, it also causes the blockage of stomata which leads to reduced transpiration and photosynthesis. The addition of charcoal and $CaCl_2$ treatment to polluted soils was able to improve the leaf area of AYB (Fig. 3), earlier findings of Mensah and Okonwu, (2016) and Viger *et al*., (2013), showed that the addition of charcoal treatment causes an increase in leaf cell expansion and photosynthesis. The results are in agreements to the findings of Burke *et al*., (2012), who also found increased leaf area due to charcoal application. The chlorophyll content of AYB was not significantly affected by the charcoal and $CaCl_2$ treatment (Table 1); this result does not correspond with the work of Younis *et al*., (2015), who recorded an increase in the chlorophyll content of spinach when biochar from cotton straw was applied. According to Hafeez *et al*., (2017), Biochar can positively affect the chlorophyll content by amplifying the performance of Photosystem II and increasing electron transport, which boosts the overall Photosynthetic output index. The highest chlorophyll content was recorded in treatment $CaCl_2$ only; this is in agreement with Xu *et al*., (2013), who recorded an increase in chlorophyll content after the application of $CaCl_2$ pre-treatment. Charcoal only treatment had the heights carbohydrate content followed by the control treatment, $CaCl_2$ pre-treatment did not improve the carbohydrate content (Table 1). Calcium is essential for cell turgor pressure, and the stability of peptic cell wall; the increase and thickening in root length is dependent on these factors (Madani *et al*., 2013). Data findings indicate that the application of $CaCl_2$ significantly increased the root length of AYB (Table 2). Xiang *et al*., (2017) indicated that the application charcoal improves the root biomass, root nodules in legumes, nutrient concentration and root morphology. Results showed calcium chloride pretreatment inhibited the emergence of AYB in a polluted soil. Charcoal and calcium chloride are good fertilizers and can improve the growth and biochemical features of African Yam Bean in crude oil polluted soil. Calcium chloride pre-treatment can promote the growth of African Yam Bean. Calcium chloride pre-treatment should not be used in polluted soils. The use of these treatments separately is encouraged than a combination of charcoal and calcium chloride in polluted soil.

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