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Growth Potentials of Cowpea (*Vigna unguiculata* L. Walp) on a Crude Oil Polluted Soil: A Screen House Experiment

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Authors' contributions

This work was carried out in collaboration between all authors. Author Okunwaye Iris designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Ogboghodo Ikponmwosa and ES managed the analyses of the study. Author OO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The study was conducted to investigate the effect of the application of cow dung to crude oil polluted soils on the growth of cowpea. It was a screen house experiment. Four rates of cow dung (0, 1, 2 and 3 g) and four rates (0, 5, 10 and 15 ml) of crude oil per 10 kg of soil were used giving a total of sixteen (16) treatment combinations. Each treatment was replicated three times, for a total of forty eight (48) pots. The rate used is equivalent to 0, 200, 400 and 600 kg/ha and 0, 1000, 2000 and 3000 litres/ha of cow dung and crude oil respectively. The experiment was laid out in a completely randomized designed. Some plant growth parameters such as plant height, number of leaves and

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leaf areas were recorded at 7DAP, 2, 4, 6, 8 and 10 weeks after planting. The plant dry matter yield was also determined. Results obtained at the end of the field experiment showed that plant height increased from 5.8 cm to 114.6 cm at the rate of 5 ml crude oil and cow dung application of no amendment. There was a continuous increase in percentage germination, number of leaves, leaf area and dry matter yield. Generally remediation for the oil contaminated soil at the end of tenth week revealed a positive correlation coefficient in the degree of remediation during the trial periods.

Keywords: Cow dung; crude oil; cowpea; growth; revegetation.

1. INTRODUCTION

Since commercial exploration of petroleum started in Nigeria in 1958, petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways.

Recently, oil companies, government agencies and individual researcher have resorted to conducting post-spill remediation measures using a combination of biological and physico-chemical treatment ameliorate soil and water quality as well as improve soil fertility status [1].

The effects of crude oil on the growth and performance of plants have been reported in many researches. These effects have been observed to occur due to the interference of the plant uptake of nutrients by crude oil and the unfavourable soil conditions due to pollution with crude oil. It has been reported that plants and soil microbes compete for the little nutrient available in soils that are not rich like that polluted with crude oil thereby suppressing the growth of plants in such soils. Generally, it is considered that in the nutrient poor soils the plants face stress for growth and development [2,3,4,5,6] hence; such soils are augmented with manure to release the plants from stress and enhancement in the growth. Merckl et al. [7] reported that addition of inorganic fertilizer in a crude oil polluted soil enhances the growth and performance of Brachiaria brizantha in crude oil polluted soil. Although, the performance of plants as reported by Merckl et al. [7] can be enhanced in crude oil polluted soil with fertilizer, it also increases the cost of crop production in crude oil polluted soil. It is therefore necessary to investigate the impact of organic manure like cow dung can make the growth of crops in crude oil polluted soil. This is because such manure is cheaper and is more affordable to farmers than the inorganic fertilizers. Cowpea is one of the most productive heat adaptive legume used agronomically in the world. It thrives best in hot moist zone but required more heat to for optimum growth. Cowpea performed well on a wide variety of soil conditions but performed best on well-drained sandy laom soil. According to Pilon-Smith [8], the ability of plants to grow quickly is one of the factors that favor bioremediation.

This study was therefore carried out to investigate whether addition of cow dung to crude oil polluted soil will enhance the growth and performance of *Vigna unguiculata* in such soil. The information obtained will serve as a good reference for using cow dung to augment soils contaminated with crude oil so as to use such soils for crop production.

2. MATERIALS AND METHODS

The pot experiments were conducted at the Faculty of Agriculture, University of Benin at the department of Soil Science and Land Management screen house. The experiment comprising of sixteen (16) treatment combinations replicated thrice, for a total of forty eight (48) buckets.

2.1 Treatments and Experimental Design

48 buckets measuring 12 litres volume capacity were used for this study. The buckets were perforated at the sides and bottom. Each bucket was filled with 10 kg of top soil equivalent to 0.10 $m^{2.}$ Soils were collected at 0 - 15 cm of soil depth, air dried, carefully cleaned by picking away all litter of leaves and roots that could decompose with time and weighed into plastic buckets. The soils were allowed to settle for two weeks, watered and treated with four rates (0, 5, 10 and 15 ml) of crude oil (bonny light blend). The crude oil was spilled on the surface of the soil in simulating what generally occurs in case of oil spills. Two weeks after crude oil treatment, four rates (0, 1, 2 and 3 gm) of air-dried, ground cow dung manure was applied to polluted soils. The cow dung manure was thoroughly mixed with the soil using hand trowel to ensure uniform

distribution within the soil. Each quantity of crude oil served as a treatment with the 0 ml treatment serving as the control.

Treated soils were watered constantly for about two weeks before planting. Four cowpea seeds were planted per bucket and later thinned to two seedlings per bucket after seven days of planting. Watering was done regularly. Unwanted weeds removed by hand picking as at when due. The experiment was a 4*4 factorial laid out in a complete randomized design.

2.2 Growth Parameters Measured

Parameters measured include plant height, number of leaves, leaf area (leave length and width), fresh weight of plant, dry matter yield and number of pod. Crop emergence (germination percentage) was taken as a percentage of the ratio of seedlings at 7 days after planting to the actual number of seeds planted. Plant height, number of leaves and leaf area were measured at two weeks intervals while dry matter yield, fresh weight of plant and number of pod were measured after harvesting plant i.e at the end of the experiment.

2.3 Determination of Total Petroleum Hydrocarbon and Pah in Crude Oil Samples

Procedure

The samples were cold-extracted in a conical flask for two hours in each case using 100 % dichloromethane according to the method of [9]. The solvent from the resultant solution was removed by means of a rotary evaporator under vacuum (pressure not greater than 200mbar) and finally by a flow nitrogen at not more than 30°C to yield the extracted organic matter (EOM).

The extracted organic matter (EOM) was analysed by capillary gas chromatography. The GC-FID system consist of a HP5890 SERIES II, Hewlett-Packard, Waldbrown, Germany GC equipped with flame ionization detector and ATLAS soft ware data processor (USA). The gas chromatographic column used was Ultra-1932530, a non- polar, fused-silica capillary column (30m × 250μm inner diameter × 0.20μm film thickness) (USA). Helium gas was used as the carrier gas at a low flow rate of 1 ml/min at a pressure of 75kpa. The injector temperature was set at 250°C, and detector temperature at 310°C. The temerature program used was; 2 minutes hold time at 250, a ramp to 13°C at 3°C/min followed by 3 min hold time, a ramp to 240°C at 7°C /min and a final ramp to 285°C at 12°C with an 8 minute hold time.

2.4 Statistical Analyses

Analysis of variance (ANOVA) was used to analyse data, using the complete randomised design and the randomised block design with replications. Mean differences among treatments were evaluated with the Tukey Least Significant Difference t-test (LSD) test.

Table 1. Concentration of PAH's in crude oil

PAH (ml/l)	Nigerian crude oil
Acenaphthene	1.072
Acenaphthylene	1.046
Anthracene	0.522
Benzo(a)pyrene	0.076
Benzo(b)flouranzthene	0.023
1,12-Benzoperylene	0.007
1,2,5,6Dibenzanthracene	0.002
Fluoranthene	0.450
Fluorene	0.284
Indeno(1,2,3)pyrene	0.002
Naphthalene	0.163
Phenanthrene	0.143
Pyrene	0.621
Benzo(k)fluorathene	BDL

3. RESULTS

3.1 Total Petroleum Hydrocarbon Content (mg/kg)

Total Petroleum Hydrocarbon from all samples pre-exposed to crude oil where all below detecting limits (BDL). The highest mean was (43.8±0.34) gotten from treatment (10 ml of crude oil, 3 g of cow dung application). Similarly, the lowest mean was recorded (6.4±0.27) at the lowest concentration of crude oil application without amendment (5 ml of crude oil, NA of cow dung application).

3.2 Germination Percentage, GP (%)

Germination percentage result showed high variability between treatments (0 ml of crude oil, NA of cow dung, 0 ml of crude oil, 2 g of cow dung, 5 ml of crude oil, 1 g of cow dung, 5 ml of crude oil, 2 g of cow dung, 10 ml of crude oil, NA of cow dung, 10 ml of crude oil, 1 g of cow dung and 15 ml of crude oil, 1 g of cow dung) and had a low germination rate of

66.7% in Treatment (15 ml of crude oil, NA of cow dung).

3.3 Plant Height

Result of plant height showed a continuous increase during the experiment. The plant height had a higher value of 114.6 in treatment (5 ml of crude oil, NA of cow dung) and low value of 5.8 in treatment (15 ml of crude oil, NA of cow dung).

3.4 Number of Leaves

The result for production of leaf in cowpea ranged from 31 (0 ml of crude oil, 1 g of cow dung) to 27 (15 ml of crude oil, NA of cow dung) as compared with other treatment. Statistical analysis for number of leaves from all the treatment groups indicated no significant difference (P>0.05) these showed that crude oil and cow dung introduced at different concentration did not significantly affect the number of leaves when compared with the control.

3.5 Leaf Area

The leaf area result had a higher value of 53.8 cm^2 in treatment (5 ml of crude oil, 2 g of cow dung) and low value of 11.0 cm^2 in treatment (15 ml of crude oil, NA of cow dung) and range from 39.3 to 53.8 cm^2 at the end of the experiment i.e. 10WAP.

3.6 Dry Matter Yield

The dry matter yield of cowpea ranged from 3.0 g to 6.1 g. it has its weight decreased from 4.1 g (0 ml, NA) in the control to 3.4 g (5 ml, NA) at the lowest crude oil application level, this was followed by an unstable increase and decreases with increasing level of crude oil application and that of increase in the rate of cow dung amendment.

3.7 Fresh Weight of Plant

The fresh weight of plant at the end of the experiment ranged from 8.6 g to 23.5 g, the plant increased from 14.2 g in the control (0 ml, NA) and decreases at the lowest level of crude oil application 8.6 g (0 ml, 3 g).

3.8 Number of Pod

Production of pods has a higher value of 4.3 g in treatment (0 ml of crude oil, 1 g of cow dung) and a low value of 0.8 g (0 ml of crude oil, NA of cow dung and 15 ml of crude oil, NA of cow dung) as compared with other treatment. Statistical analysis for result of number of pod obtained after harvesting indicates there were no significant differences (P>0.05) between the control and treatment groups.

4. DISCUSSION

The increased percentage germination associated with the high rates of cow dung amendment as pollution level increased may be attributed to nutrient addition.

Table 2. Effect of application of cow dung to crude oil polluted soil on plant height (CM)

Treatments	7DAP	2WAP	4 WAP	6 WAP	8WAP	10 WAP
0(NA)	9.4abC	16.0aC	24.3aBC	44.7aB	74.1aA	93.5aA
0(1)	9.9abD	14.5aD	39.2aC	65.5aB	90.7aA	105.5aA
0(2)	12.8abC	17.7aC	30.3aBC	54.7aB	84.8aA	102.8aA
0(3)	11.4abD	18.2aD	31.2aCD	57.7aBC	81.7aAB	101.3aA
5(NA)	12.2aD	19.4aD	38.0aCD	66.0aBC	94.7aAB	114.6aA
5(1)	11.5aD	16.7aCD	38.2aCD	53.2aBC	83.0aAB	100.2aA
5(2)	12.2aC	17.6aC	33.0aBC	56.8aABC	80.5aAB	99.3aA
5(3)	12.5aC	17.6aC	34.2aBC	61.8aABC	88.0aAB	104.7aA
10(NA)	8.9abE	16.3aDE	28.2aD	50.2aC	77.3aB	96.3aA
10(1)	10.7abB	16.1aB	47.8aAB	66.8aAB	90.8aA	100.7aA
10(2)	11.5abD	18.9aD	42.5aC	68.5aB	93.5aA	107.0aA
10(3)	9.2abD	15.9aCD	42.2aBCD	59.8aABC	86.3aAB	100.7aA
15(NA)	5.8bC	10.7aBC	29.7aBC	48.7aABC	72.8aAB	96.7aA
15(1)	10.9bB	16.3aB	33.1aB	57.7aAB	83.8aA	100.7aA
15(2)	10.0bC	17.0aC	33.7aC	60.8aB	88.8aA	105.2aA
15(3)	10.9bC	17.0aC	33.5aC	65.5aB	92.0aAB	105.0aA

[a-b] *Different letters in the same column indicate significant difference (P<0.05)*
[A-E] *Different letters in the same row indicate significant difference (P<0.05)*

Table 3. Effect of application of cow dung to crude oil polluted soil on the number of leaves

Treatments	2WAP	4 WAP	6 WAP	8 WAP	10 WAP
0(NA)	5.0aE	11.8aD	20.5aC	26.3aB	29.5aA
0(1)	5.0aC	14.5aBC	24.2aAB	29.7aA	31.4aA
0(2)	4.5aC	12.5aBC	20.2aAB	25.5aA	28.0aA
0(3)	5.3aC	12.5aB	23.7aA	27.7aA	29.3aA
5(NA)	5.0aD	14.5aC	23.5aB	28.2aA	29.7aA
5(1)	5.0aB	11.8aB	21.2aA	26.2aA	27.2aA
5(2)	6.0aD	14.3aC	24.0aB	29.5aA	28.3aA
5(3)	6.0aC	13.2aBC	22.5aAB	30.3aA	27.2aA
10(NA)	5.0aD	11.5aC	20.2aB	25.2aA	28.3aA
10(1)	5.5aC	11.7aBC	20.3aAB	27.8aA	29.0aA
10(2)	5.5aB	12.5aB	23.3aA	28.8aA	30.3aA
10(3)	6.0aC	12.0aC	20.2aB	26.2aAB	29.0aA
15(NA)	4.3aD	11.5aC	19.7aB	25.8aA	27.0aA
15(1)	5.0aD	13.5aC	23.2aB	28.5aA	27.8aA
15(2)	5.2aD	12.3aC	20.7aB	26.7aA	28.0aA
15(3)	5.0aB	13.5aB	22.5aA	27.7aA	27.5aA

[a] Same letters in the same column indicate no significant difference (P>0.05)
[A-E] Different letters in the same row indicate significant difference (P<0.05)

Table 4. Effect of application of cow dung to crude oil polluted soil on leaf area (cm^2)

Treatments	2WAP	4 WAP	6 WAP	8 WAP	10 WAP
0(NA)	16.6aB	24.9aAB	30.7aAB	34.9aAB	39.3aA
0(1)	19.4aA	30.3aA	36.7aA	41.6aA	46.0aA
0(2)	20.6aA	26.8aA	34.5aA	39.7aA	44.5aA
0(3)	22.1aA	29.0aA	36.5aA	41.5aA	45.1aA
5(NA)	20.8aA	32.6aA	38.5aA	43.1aA	48.3aA
5(1)	17.0aA	33.5aA	40.5aA	46.6aA	46.9aA
5(2)	19.9aB	36.4aAB	42.6aAB	51.9aA	53.8aA
5(3)	20.2aA	34.4aA	40.6aA	45.9aA	50.7aA
10(NA)	17.7aA	26.6aA	33.2aA	39.2aA	46.1aA
10(1)	19.5aA	25.3aA	30.3aA	35.0aA	41.4aA
10(2)	20.3aB	28.9aAB	34.9aAB	50.1aA	51.5aA
10(3)	17.0aA	27.2aA	34.3aA	38.8aA	44.4aA
15(NA)	11.0aB	21.3aAB	27.5aAB	39.1aAB	39.3aA
15(1)	18.1aB	32.2aAB	46.5aA	49.3aA	49.5aA
15(2)	15.5aC	25.0aBC	30.9aABC	44.3aAB	44.8aA
15(3)	18.6aB	39.6aA	37.4aAB	51.3aA	51.3aA

[a] Same letters in the same column indicate no significant difference (P>0.05)
[A-C] Different letters in the same row indicate significant difference (P<0.05)

Plants have been reported by Onuh et al. [10] to grow better with adequate soil nutrients even in the face of crude oil pollution which was also observed in this experiment. When soils were amended with cow dung plant height rose significantly with application rate 5 ml crude oil and NA cow dung. This may be due to the fact that upon mineralization of the added crude oil, nutrient was released to the soil, giving rise to increase in the plant height and the level of crude oil pollution may have been too low in concentration to cause harm to the plant.

Results from the number of leaves on each plants indicates no statistically significant difference (P>0.05) between the control and the treatment groups, Indicating no effect of crude oil or amendment on the growth of leaves. This was in conformity with the work of [11] who discovered that crude oil at low concentrations are essential micro-nutrients for plants, but at high concentrations, they may cause metabolic disorders and growth inhibition.

The production of pods as well as flowering was not obviously affected. The production of pods and flowering occurred at about the same period. Results from production of pods on each plant indicates no statistically significant differences (P>0.05) between the control and the treatment groups indicating no effect of crude oil or amendment on the production of pods. [12] observed that small amount of hydrocarbon in substrates can enhance growth media and indirectly growth characteristics.

The increased in the dry matter content observed in this study could be attributed to continuous growth of the plant. The addition of cow dung to soils contaminated with crude oil led to increase of dry matter Content of cowpea grown in the soils.

Table 5. Effect of cow dung application to crude oil polluted soil on germination percentage, dry matter yield of cowpea, fresh weight of plant of cowpea and number of cowpea pod

Treatments	Germination %	Dry matter yield	Fresh weight of plant	Number of pod
0(NA)	100a	4.1a	14.2a	0.8a
0(1)	75a	5.4a	20.8a	4.3a
0(2)	100a	5.9a	21a	3.0a
0(3)	91.7a	6.1a	23.5a	2.7a
5(NA)	91.7a	3.4a	8.6ab	2.0a
5(1)	100a	5.5a	19.8ab	2.3a
5(2)	100a	3.9a	14.4ab	2.8a
5(3)	91.7a	5.2a	17.6ab	2.8a
10(NA)	100a	3.3a	11.2ab	1.3a
10(1)	100a	3.8a	13.9ab	1.8a
10(2)	91.7a	3.8a	11.5ab	2.3a
10(3)	83.3a	3.8a	17.1ab	1.3a
15(NA)	66.7a	3.7a	11.2b	0.8a
15(1)	100a	3.3a	11.8b	1.2a
15(2)	91.7a	4.2a	14b	1.3a
15(3)	91.7a	3.0a	10.9b	1.2a

[a] Same letters in the same column indicate no significant difference (P>0.05)
[a-b] Different letters in the same column indicate significant difference (P<0.05)

Table 6.Total petroleum hydrocarbon content

	Total Petroleum Hydrocarbon (TPH)		
Treatments	**TPH (Pre-exposed Soil)**	**TPH (Before Planting)**	**TPH (After Harvesting)**
0(NA)	BDL	BDL	BDL
0(1)	BDL	BDL	BDL
0(2)	BDL	BDL	BDL
0(3)	BDL	BDL	BDL
5(NA)	BDL	17.6±084	6.4±0.27
5(1)	BDL	11.3±0.25	BDL
5(2)	BDL	10.4±0.26	BDL
5(3)	BDL	10.5±0.36	BDL
10(NA)	BDL	32.9±0.36	13.24±0.03
10(1)	BDL	26.4±4.58	BDL
10(2)	BDL	20.2±5.45	BDL
10(3)	BDL	43.8±0.34	BDL
15(NA)	BDL	30.4±5.72	14.22±0.05
15(1)	BDL	28.9±5.72	BDL
15(2)	BDL	28.9±0.64	BDL
15(3)	BDL	28.1±0.09	BDL

Key: NA = No Amendment; 1= 1 g of cattle dung; 2 = 2 g of cattle dung; 3 = 3 g of cattle dung; 0 = No crude oil; 5 = 5 ml crude oil; 10 = 10 ml crude; 15 =15 ml crude oil

The addition of cow dung led to increase in the leaf areas, there was no statistically significant difference (P>0.05) between the control and the treatment groups, Indicating no effect of crude oil or amendment on the growth of plant. Pair wise comparison of the leaf area as the week progressed indicates statistically significant differences (P<0.05) between weeks, thus indicating steady growth within the period of the experiment. This is in agreement with the report of Christo et al. [13].

There were statistically significant difference (P<0.05) between the control and the treatment groups. These reports are in line with those of [14], who determined significant increase in plant biomass with different crude oil concentrations.

5. CONCLUSION

This study provides valuable and useful information concerning the effect of cow dung application to crude oil polluted soils, as a bioremediation technology, in the view of improving the growth and performance of cowpea (*Vigna unguiculata* on). The obtained information will serve as a good reference for using cow dung to improve the soil contaminated with crude oil, to its further exploitation for crop production. Cowpeas are good for revegetation. Remediation for the oil contaminated soil at the end of tenth week revealed a positive correlation coefficient in the degree of remediation during the trial periods.

The process involving the use of organic manure has been considered for the potential for biodegradation and biotransformation of petroleum products, which indicates that bioremediation methods are more efficient and cheaper than chemical processes. The proposed method can be applied on a large scale because the manure is environmentally friendly and also, it has been observed to promote the bioremediation of hydrocarbons.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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