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Effect of Escravos Light Crude Oil on Cowpea (*Vigna unguiculata*) Germination and Growth

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Abstract: ***This study was carried out to investigate the effect of Escravos light crude oil on cowpea germination and growth. Two varieties (white and brown) of cowpea (Vigna unguiculata) seeds were used and studies on the effect of cowpea nodulation were investigated on soils treated with various concentration of Escravos light crude oil. The oil was mixed with the soil, watered to keep it moist and left for 3 days for percolation of the oil. Control was set up with uncontaminated soil and each experiment was set in duplicates and watered every morning with 10mls of water. The plants were observed for the rate of growth, shoot length, leaf colour, width of foliage and root nodulation. After 42 days of planting, the plant was harvested; roots were carefully removed and examined for nodulation. Nodules were counted and diameter measured. It was observed that the soil contaminated with hydrocarbon inhibited growth and where there was growth, decreased shoot length. Nodules were also found to be absent in the contaminated soil.***

Keywords: Escravos light crude oil, Cowpea, Nodulation, Plant, Germination

1. Introduction

The impact of crude oil spillage and discharge on the ecosystem as a result of oil exploration activities is an obvious problem of environmental concern [1]-[2]. Generally, studies on crude oil have revealed that it has serious deleterious effects on soils [3]-[5], plants [6]-[7], aquatic life [8]-[9] and even organisms such as the macrobenthic invertebrates [10]. When oil is spilled intentionally or unintentionally, the immediate and remote environment, including the soils, is contaminated. Oil pollution due to spill could take place in water or on land. Crude oil pollution on land depends on a number of factors which include; the permeability of the soil, adsorption properties of the soil and the partition coefficient [11]. The extent of contamination depends on the chemical composition of the contaminant and the properties of the soil [12]. Regardless of the source of contamination, once hydrocarbons come into contact with the soil, they alter its physical and chemical properties. The degree of alteration depends on the soil type, the specific composition of the hydrocarbon spilled and the quantity spilled.

Cowpea (*Vigna unguiculata*) is a warm season legume grown in tropical and subtropical regions of Africa, Asia and the Americas. It is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times [13]. Cowpea seeds vary considerably in size, shape and colour. Cowpeas are susceptible to a wide range of pests and pathogens that attack the crop at all stages of growth [14]. Microbial infections of plants and seed could be genetic, cultural or chemical. Escravos crude oil is produced in Nigeria and as long as the exploration and exportation continue, the incidence of oil spills and hydrocarbon pollution will continue to be anticipated and studied. Oil spillage takes place ever so frequently and this has serious implications for terrestrial and aquatic ecosystem. The objective of this study focused on the effect of Escravos light crude oil on *Rhizobium* and nodule formation.

2. Materials and Methods

2.1. Sample collection

Soil sample (loamy soil) was collected at planting time. One hundred and forty gram of the soil were weighed into plastic cups, these cups were then grouped according to the experimental setups. Two varieties of cowpea (brown and white) used for this study were purchased from Kuto Market in Abeokuta, Ogun state, Nigeria**.**

2.2. Viability Test

Cowpeas used were checked for viability. The viability rate was determined by planting randomly selected seeds in plastic cups containing one hundred and forty gram garden soil. The experiment was carried out in duplicates with two seeds in a cup and watered every morning with Cowpeas used were checked for viability. The viability rate was determined by planting randomly selected seeds in plastic cups containing one hundred and forty gram garden soil. The experiment was carried out in duplicates with two seeds in a cup and watered every morning with 10mls of water. Seeds which germinated with green foliage were scored as viable.

2.3. Planting

One hundred and forty gram garden soil was weighed into slightly perforated plastics cups and was uniformly mixed with various concentrations of Escravos light crude oil. Each cup was contaminated with a known volume of Escravos light crude oil. The treatment volumes added to the soil were 1, 2, 3, 4, 5 and 6ml with the control not contaminated. Each soil sample was watered with 10mls of water to make it moist and mixed with a plastic spatula. This was left for some time to ensure percolation of the crude oil. After three days, two cowpea seeds were planted in each cup. A control was set up with unpolluted soil; all the experimental cups were then taken to the green house and watered every morning with 10mls of water. The plants were observed for the rate of growth, shoot length, leaf colour, width of foliage and root nodulation. After 42 days of planting, the plant was harvested. Roots were carefully removed and examined for nodulation. Nodules were counted and diameter measured.

3. Result and Discussion

3.1. Viability Rate

Nearly all the cowpea seeds were viable. They germinated within 24 hours except the seeds in one of the cups contaminated with 1ml and 4 ml of the hydrocarbon both belonging to the white variety of cowpea indicating that the seeds were not viable.

3.2 Physical Investigation

3.2.1. Rate of Growth

All the soil samples were treated with varying concentrations of Escravos light crude oil except the control. The seeds which sprouted within 24 hours were scored as viable.

Table 1: The shoot length and leaf colour for Brown and White Cowpea varieties grown in polluted soil at the 7th day

Quantity of contaminant (µg/ml)	*Average shoot length (cm)*		*Leaf colour*	
	Brown	*White*	*Brown*	*White*
control	19.75±0.01	17.38±0.01	Greenish yellow	Green
1ml	14.50±0.01	17.75	Green	Green
2ml	13.63±0.01	17.25±0.01	Greenish yellow	Green
3ml	15.50±0.01	16.50±0.01	Greenish yellow	Greenish yellow
4ml	15.88±0.01	17.03	Green	Green
5ml	15.88±0.01	13.13±0.01	Green	Green
6ml	14.88±0.01	17.25±0.01	Green	Green

Note: The value are means ± standard error of means of three replicate value

It was observed that the plants were all growing at the same rate at the first few days of planting since the contaminated soil had no effect on the plants yet.

Table 2a: The shoot length in Brown (CB) and White (CW) Cowpea plant grown in polluted soil

Days of planting	Cowpea variety	Average shoot length (cm)			
		Control	1ml	2ml	3ml
5	CB	6.50±0.11	6.30±0.22	5.90±0.11	5.83±0.05
	CW	6.20±0.27	7.58	8.11±0.09	6.80±0.04
10	CB	19.75±0.24	14.50±0.16	12.50±0.25	10.50±0.03
	CW	18.25±0.17	16.66	15.53±0.05	14.00±0.09
15	CB	21.00±0.35	15.25±0.09	10.15±0.31	8.66±0.34
	CW	19.98±0.02	13.03	7.15±0.22	7.05±0.17
20	CB	21.60±0.17	16.12±0.10	7.12±0.14	5.45±0.57
	CW	20.55±0.12	10.05	2.20±0.09	1.15±0.33
25	CB	22.15±0.44	16.15±0.22	4.00±0.15	2.70±0.11
	CW	21.55±0.23	8.15	NG	NG
30	CB	22.90±0.18	16.40±0.03	3.12±0.25	NG
	CW	22.74±0.02	5.07	NG	NG
35	CB	23.80±0.11	16.90±0.01	0.70±0.17	NG
	CW	24.73±0.31	3.23	NG	NG
40	CB	24.28±0.70	17.5±0.02	NG	NG
	CW	26.15±0.58	0.95	NG	NG

Note: The values are means ± standard error of means of three replicate values; NG: No observed growth

The shoot length was measured at five days interval, by the 13th day; the hydrocarbon contaminated soil had started affecting the cowpea growth and development. As the planting days proceeded the effect of the hydrocarbon were more pronounced on the soil treated with higher concentration than those treated with lower concentration. The effects were more pronounced on white cowpea than the brown ones. By the 39th day, the shoots were off in most plants as a result of the contaminated soil affecting plant development. After six weeks at the time of harvesting, only control for white and brown variety and one of the plants in the cup containing soil polluted with 1ml of escravos light crude oil survived. However, one of the control for white cowpea showed climbing growth. Leaves colour for both control were green, while brown cowpea polluted with 1 ml escravos light crude oil had yellow leaves.

Table 2b: Average shoots length in Brown (CB) and White (CW) Cowpea plant

Days of planting	Cowpea variety	Average shoot length		
		4ml	5ml	6ml
5	CB	5.78±0.17	6.16±0.09	5.85±0.15
	CW	6.68	6.90±0.05	7.00±0.07
10	CB	11.88±0.01	13.88±0.03	12.80±0.13
	CW	13.25	12.15±0.01	14.15±0.21
15	CB	8.70±0.01	7.70±0.21	6.23±0.01
	CW	5.98	2.15±0.12	0.90±0.42
20	CB	4.15±0.41	2.60±0.11	1.00±0.23
	CW	0.90	NG	NG
25	CB	1.90±0.01	NG	NG
	CW	NG	NG	NG
30	CB	NG	NG	NG
	CW	NG	NG	NG
35	CB	NG	NG	NG
	CW	NG	NG	NG
40	CB	NG	NG	NG
	CW	NG	NG	NG

Note: The values are means ± standard error of means of three replicate values; NG: No observed growth

Table 3: Nodule count and diameter of White and Brown variety of Cowpea

Treatment	Nodule count	Diameter of nodules
Control White cowpea	15	0.300
Control brown	8.5	0.065

From this study, it was observed that escravos light crude oil had a considerable effect on growth, development and nodule formation in cowpea as the control showed a significant difference from the contaminated plants. Germination was observed within a week of planting which corroborated with the findings of Craufurd *et al.* [15]. It was observed that the plants were all growing at the same rate at the first few days of planting since the Escravos light crude oil used had no effect on the plants. By the 10th day, the contaminated soil had started having effect on the plants growth and development as observed with the shoots bending and leaves drying off with the effect on the higher concentration more pronounced. As the planting days proceeded, it was observed that growth ceased completely in some of the plants and by the 30th day, most of the plants had their shoots and roots off. These treatments had a negative effect on development compared to the control and the effect progressed with increase in concentration of the hydrocarbon. This is because when petroleum hydrocarbon contaminate soil, the carbon: nitrogen (C: N) ratio of the soil is altered, the added carbon stimulates microbial numbers but causes an imbalance in the carbon: nitrogen (C: N) ratio which may result in immobilization of soil nitrogen by the microbial biomass, leaving none available for plant growth [16]**.** In terms of nodulation the control showed a significant decrease from the contaminated ones since the most problematic environments for *Rhizobia* are marginal land with low rainfall, extremes of temperature, acid soils of low nutrient status and poor water holding capacity. Six weeks after planting, the plants were harvested; roots were carefully removed and examined for nodulation. Only the control for white and brown cowpea plants and one of the brown varieties treated with 1ml concentration of the crude oil remained. Comparing the nodule size and number of nodules on both plants; the white variety had bigger and higher number of nodules than the brown variety. No nodule formation in the brown variety contaminated with 1ml, this is because though the plant did not die off by the alteration of the soil hydrocarbon there was absence of *Rhizobium* which fixes nitrogen and develops nodules on roots of leguminous plants. However the result of this study shows a relation with previous studies on the effects of diesel fuel on common vetch plants by Adam and Duncan [16].

4. Conclusion

It can be concluded that Escravos light crude oil had adverse effect on cowpea development and nodulation but the effect is more pronounced on white cowpea seeds than brown ones. Cowpea being an important socio-economic plant including its importance in improving fertility of the soil through symbiotic relation with *Rhizobium* can be disrupted when petroleum hydrocarbon contaminate soil on which it is grown. Therefore, cowpea growth is inhibited on poorly contaminated soils. Clean up of hydrocarbon contaminated ecosystem should be approached in a costeffective and environmentally friendly manner. Bioremediation as one of the most acceptable technology that can meet up with the regulations that govern clean up of oil-polluted sites should be encouraged.

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