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Phytochemical Effect of Petroleum on Peanut (*Arachis hypogea*) Seedlings

[1]B.O. Peretiemo-Clarke and [2]F.I. Achuba
[1]Department of Chemistry, [2]Department of Biochemistry,
Delta State University, P.M.B. 1 Abraka, Nigeria

Abstract: Investigation were carried out on the effect of various concentrations (0.00, 0.25, 0.50, 1.00, 1.50 and 2.00%), of Odidi well specie crude oil on the growth and organic constituents of peanut (*Arachis hypogea*) seedlings. Levels of total soluble sugars, proteins and free amino acids were found to be significantly ($p<0.05$) higher in plants grown in hydrocarbon polluted soil, than those grown in control soil. However, the chlorophyll content was significantly ($p<0.05$) lower in plants grown in petroleum treated soil relative to control plants. The growth parameters, such as seed germination, leaf areas, stem length and root length reduced as the concentration of petroleum increased. These finding seem to suggest that crude oil induced environmental stress in seedlings.

Key words: Peanut, pollution, environmental stress, hydrocarbon

INTRODUCTION

Nigeria is a major petroleum producing country thereby predisposing the environment to the drastic effects associated with its exploration and exploitation (Ifeadi and Nwankwo, 1987; Amadi *et al*.,1993; Jessup and Leighton, 1996; Siemiatycki *et al*., 1987).

The constituents of crude oil are complex and include aliphatic, alicyclic and polyaromatic hydrocarbon as well as oxygen, nitrogen and sulphur-containing substances (Westlake, 1982).

Chemical changes in plants associated with contaminated environment have been established by an array of scientific investigation. These include heavy metal accumulation in lower plants (Parakinen *et al*., 1978; Hertz *et al*., 1984; Makinem, 1987; Ruhling *et al*., 1987); reduction in chlorophyll, protein and carotenoids (Agrawal, 1992) and increase in total amino acids (Rowland *et al*., 1988; Schmeink and Wild, 1990).

Alteration in chemical composition is one of the methods of monitoring effect of hydrocarbon contaminated soil in plants (Achuba, 2006). The effect of hydrocarbon contaminated soil at sublethal concentration on the chemical composition of germinating peanut (*Arachis hypogea*) seedlings has not been studied. The aim of the present study, therefore is to investigate the effect of Whole Crude Oil (WCO) on the growth and composition of organic compounds of peanut seedlings.

MATERIALS AND METHODS

The crude oil was the Odidi well specie (specific gravity 0.8708) obtained from Shell Petroleum Development Company (SPDC) Nigeria Ltd., Warri, Nigeria.

Dry brown seeds of *Arachis hypogea* were obtained as a single batch from a local market in Abraka, Delta State, Nigeria. Seed viability was determined by flotation. All seeds that floated on water were discarded and those that remained at the bottom of water were deemed viable.

The sandy soil (sand 84%, salt 5.0%, clay 0.4% and organic matter 0.6%, pH 6.1) was obtained from the nursery beds of Delta State University farm project. Eight hundred grams of soil was added to each of 100 small planting bags (1178.3 cm^3, 15 cm deep) and divided into six groups of 20 replicates. Groups 1-5 contained 0.25, 0.5, 1.0, 1.5 and 2.0% (v/w), respectively of crude oil while group six served as control (0%).

Five seeds were planted in each test bag to an approximate depth of 2 cm and kept under partial shade. During the experiment 80 cm^3 of water was supplied to the set up when needed to keep the soil moist. Germination (indicated by the appearance of epicotyls above soil level) was recorded at 4 days interval up to 12 days. Seeds which failed to sprout after 12 days, were regarded as not germinated.

Three leaves from each of the 12 day old plants were cut, separately weighed and homogenized in 80% acetone.

Corresponding Author: F.I. Achuba, Department of Biochemistry, Delta State University, P.M.B. 1 Abraka, Nigeria

The extract was centrifuged at 3500 rpm at 0°C for 20 min. The solutions were diluted to known volumes and the content of the chlorophyll determined by the method of Lichtenthaler (1987).

The plants from the five treatments were analyzed for changes in total protein, amino acids and sugar. The leaves of the plants were homogenized with chloroform/methanol (2:1 v/v), then filtered and the residue was re-extracted, sugar was estimated by the method outlined by Tietze (1982). From another portion of the 70% methanol extract, the organic solvent was removed under pressure and the remaining aqueous portion diluted with 0.01 N HCl and used for the determination of total free amino acid by ninhydrin after deproteination with 5% TCA. Total protein was determined by the method of Lowry *et al.* (1951).

The experimental design was completely randomized. Analysis of variance was carried on all data and the Duncan Mutiple Range Test (DMRT) used to compare the means, $p<0.05$ were taken as significantly different.

RESULTS

The concentration of chlorophyll decreased generally as the concentration of petroleum in soil increased (Table 1). The decrease was significant at concentration of 0.05 and 2.00% oil in the soil compared to control. (Table 1) However, the concentration of the total sugar component increased at percentage concentrations of 1.00 and 2.00%. The protein constituent increased with increasing concentration of petroleum hydrocarbon in soil.

The rate of germination of seedlings was generally reduced with increasing concentration of petroleum hydrocarbon and this was statistically significant at a concentration of 1.00% compared with control (0.00%). The leaf area did not vary consistently with the concentration of petroleum, but there was a significant decrease at 2.00% concentration compared to control. The effect on stem length was also inconsistent with marked decrease at 2.00% concentration. However, there was a significant ($p<0.05$) decrease in root length at 2.00% concentration of petroleum in soil relative to control (Table 2).

After 12 days the leaf area decreased as concentration increased and was significant at higher concentration 2.00%. The stem length also decreased but not in a concentration dependent manner. The decrease was statistically significant at 2.00% concentration compared to control. The root length also reduced with increased concentration. Root length was significantly lower at 1.50% concentration (Table 3).

Table 1: Effect of petroleum hydrocarbon on the organic constituents of peanut (*Arachis hypogea*) seedlings

Crude concentration (%)	Chlorophyll (mg g^{-1})	Total sugar (mg g^{-1})	Protein (mg g^{-1})	Amino acid (mg g^{-1})
0.00	402.70±2.48	66.30±4.14	17.00±0.82	6.70±0.29
0.25	300.00±16.30	55.00±5.00	17.70±0.47	7.70±0.37
0.50	280.00±8.20	45.70±2.62	19.70±1.25	9.30±1.25
1.00	330.00±8.20	55.70±1.56	24.00±1.63	13.701.90
1.50	ND	ND	ND	ND
2.00	268.30±6.20	74.70±4.19	34.00±1.63	11.00±0.82

Results are expressed as mean±SD of determinations from five seedlings ND = Not Determined

Table 2: Effect of crude oil on the growth of peanut (*Arachis hypogea*) seedling after 8 days

Crude concentration (%)	Seed germination (%)	Leaf area (cm^2)	Stem length (cm^2)	Root (cm)
0.00	88.00	1.54±0.87	3.46±0.39	8.96±5.54
0.25	72.00	2.64±0.43	4.10±0.20	8.10±1.65
0.50	64.00	2.14±0.87	4.14±0.14	9.53±3.97
1.00	44.00	1.45±0.06	2.70±1.50	9.35±1.25
1.50	56.00	1.87±0.22	3.40±0.37	10.53±0.47
2.00	60.00	0.73±0.14	1.15±0.55	5.70±0.20

Results are expressed as mean±SD of five determinations from five seedlings

Table 3: Effect of crude oil on the growth of peanut (*Arachis hypogea*) seedlings after 12 days

Crude concentration (%)	Leaf area (cm^2) Mean±SD	Stem length Mean±SD	Root length (cm) Mean±SD
0.00	6.17±0.33	11.36±0.48	18.90±1.69
0.25	4.15±1.51	8.12±1.48	17.70±1.80
0.50	3.5±0.65	7.64±0.52	18.82±1.44
1.00	3.66±1.45	7.78±1.84	18.65±4.93
1.50	3.38±0.68	8.62±0.88	14.06±0.52
2.00	2.68±0.44	7.63±0.21	15.10±0.86

Results are expressed as Mean±SD of determination from five seedlings

DISCUSSION

Petroleum mediated stress in plants has been reported earlier (Achuba, 2006). The result of the present study (Table 1) shows that the peanut seedlings growing in hydrocarbon contaminated soil are under environmental stress as indicated by the dose dependent significant ($p<0.05$) increase in protein, amino acid and sugar contents relative to control. Stress-induced increase in sugar levels in plants have been reported to be the effect of changes in amyloplast membrane and electrolyte leakage (Hayashi *et al*., 1992). Similarly, increase in protein concentration is reported in plants under sulphur dioxide (Murray *et al*., 1992). The increase in protein level is consistent with the fact that one of the ways plants use to detoxify oxides of nitrogen in leaves is through the synthesis of protein and amino acids (Kemble and McPherson, 1954; Murray *et al*., 1992). Therefore, the increase in amino acids and protein in this study could be due to the crude oil borne oxides of sulphur and nitrogen (Anoliefo, 1991). Reduction in chlorophyll content has been an indication of environmental contamination (Agrawal, 1992). This may explain the lower level of chlorophyll in plants exposed to crude oil contamination soil.

There was significant growth depression by crude oil at various levels of soil contaminations. Similar observations ranging from decreased germination to retarded growth were reported by earlier studies (Anoliefo, 1991; Smith *et al*., 1989). Growth depression by petroleum hydrocarbon was earlier attributed to decrease in cell size and division (Achuba, 2006). The responses of the plant do not follow a definite pattern and seem inconsistent. Similar inconsistent effects of petroleum hydrocarbon on plant growth have been reported by various authors (Achuba, 2006; Edema and Okoloko, 1997; Malallah *et al*., 1996). Previous observation indicated that crude oil at certain concentration can stimulate plant growth (Bamidele and Agbogidi, 2000; Edema and Okoloko, 1997; Achuba, 2006). This has been attributed to the presence of substances such as napthenic acids (Baker, 1970). The toxicity of petroleum hydrocarbon at higher concentrations has been linked to displacement of nutrients and nutrient linkage (Amadi *et al*., 1993); reduction in available phosphorus and total nitrogen (Baker-Coker and Ekundayo, 1995) and intereference with soil chemotaxis by crude oil (Rosenburg *et al*., 1992), culminating in growth retardation (Traven, 1992).

It can be conclude that crude oil is toxic to plant. However, the toxicity is inconsistent. Therefore, there is the need to explore which of the constituent of crude oil are more toxic and at which concentrations.

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