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Effects of Different Crude Oil Fractions on Total Chlorophyll, Glucose and Starch Content of Maize *(Zea mays)*

Olubodun Stella O.[1] and Eriyamremu George E.[2]*

[1] Department of Science Laboratory Technology, Edo State Institute of Technology and Management, Usen, Edo State, Nigeria*

[2] Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin. Edo State, Nigeria

Abstract

This study was carried out to determine the effects of different crude oil fractions on total chlorophyll content, glucose and sugar content of Zea mays. The maize seeds were planted in polythene bags in the Department of Biochemistry, Faculty of Life Sciences Laboratory garden. A total of 300 bags, each containing 500g of sandy loam soil, with no known history of crude oil contamination were used for this study. 270 bags were used for planting test samples of 2%, 5% 10% crude oil fraction while 30 bags served as control for planting maize. The experiment lasted for a period of about one month. The seedlings were harvested after 7, 14, and 21 days post germination and the biochemical analysis of the roots was determined in the Department of Biochemistry and School of Basic Medical Sciences in University of Benin City, Nigeria. The data obtained were subjected to descriptive statistic of variance analysis. Crude oil at all levels of contamination and for all fractions on soil, significantly reduced total chlorophyll content in the leaves and starch contents in the roots of maize seedlings. In contrast, the glucose content significantly increased in the roots for all fractions at all levels of contamination of the soil. The significance of the increasing glucose content in the maize plant as a result of crude oil contamination is discussed.

Keywords: Crude oil fractions, glucose content, starch content, total chlorophyll.

Introduction

Crude oil is very important in Nigeria as it is presently the major source of income for the country as well as major source of fuel (energy). Crude oil has been shown to affect plant growth and development by inducing biochemical, physiological and morphological changes in the cells and tissues of different plant species (1, 2 and 3). Research on the effects of crude oil on some plants has been done, with little or no information on the effects of different fractions of crude oil on total chlorophyll, glucose and starch content in maize plants to date. Several reports on the effects of crude oil on plant growth have revealed various physiological and morphological aberrations in germination and growth rates (1 and 4) with reduction in plant height, number of leaves (2 and 5) and altered enzyme activity (2 and 3).

Maize (*Zea mays*) or corn, a member of *Gramineae* family, is one of the main cereal staples in Nigeria. It is cultivated in every important agricultural area in the world (6). The Niger-Delta area of Nigeria where most of Nigeria's crude oil industrial activities is rampant is no exception. The nutrient content of the plant varies among varieties, various plant parts and also changes with age and stages of development. However, the content of the seeds include a protein content of about 9.4 g/100g, fat content of 4.3 g/100g, fiber content of 1.8 g/100g, carbohydrate content of 74.4 g/100 and water content of 10.6 g/100g fresh weight (7). Apart from being a source of food and feed, it has other important uses which range from its use for fuel or cosmetics (8) to being pharmacological (7). Different parts of the plants are used in the treatment of strangury, dysuria and gravel. They are cholagogue, demulcent and diuretic (8). They are also used in the treatment of diabetes mellitus, cystitis, gonorrhea, cancer, tumors and warts because research have shown that it contains the cell-proliferant and wound-healing substance allantoin, used widely in herbal medicine to speed up healing process (7 and 8). In spite of being such an important plant in Nigeria, the research information documenting the effects of different crude oil fractions on total chlorophyll, glucose and starch content in maize is scarce. Abiotic and biotic stresses cause alterations in the physiological processes of plants and decrease their productivity.

Since crude oil and other environmental pollutants have been shown to alter physiological processes of plants and decrease productivity, the aim of this research was to determine the effects of different fractions of crude oil on developing seedlings of maize. The objective was to analyze the changes in total chlorophyll content, glucose and starch content making comparison in control and test plants at different growth stages.

** Corresponding Author: sabukadi@yahoo.com; Tel: +2348023411948*

Material and Methods

Study location: The *ex situ* study was carried out at the University of Benin, Benin City, Edo State, Nigeria from the month of March, 2009. The soil from an uncultivated land with no known crude oil contamination in Edo State was collected. The region is made up of flat plains with sandy loam soil characterized as coastal plain sand. The region experiences moderate rainfall and moderate humidity for most part of the year. Temperature and humidity remain relatively constant throughout the year. The climate is marked by two distinct seasons: the dry season and the rainy season. The area is characterized by tropical equatorial climate with mean annual temperature of 32.8 °C and annual rainfall of about 2673.8 mm. The natural vegetation is dense tropical rainforests with swamp forest in some areas.

Soil Sampling and Pre-treatment: Top soil (0 – 20cm depth) sample was collected from an uncultivated land without known crude oil contamination in Edo State. Holes were dug at five different points within the land to a 20cm depth each using plastic spade. The soil samples was collected into polythene bags and taken to the laboratory. A composite of all the samples was made by mixing thoroughly equal amounts of soil from each point.

Soil treatment: The composite soil was weighed into 300 polythene bags such that each bag contained 500g soil. The composite soils were treated with either distilled water (control); whole crude (WC), water soluble fraction (WSF) of the crude oil; or with the water insoluble fractions (WIF) of the crude oil in the laboratory. The soil in the bags contaminated with whole crude (WC), water soluble fraction (WSF), and water insoluble fractions (WIF) were mixed thoroughly in their respective polythene bags containing 500g top soil with the aid of a plastic spade. Soil of 500g was treated with 10ml, 25ml and 50ml of crude oil to obtain 2, 5 and 10% v/w crude oil contamination (Table 1). In each bag, three (3) viable maize seeds were planted. Equal amounts of seeds that germinated were harvested at day 7, day 14 and day 21 and their leaves and radicle taken for analysis.

Plant Materials: Maize (*Zea mays*) seeds identified as Dmr-Esr-w, by the Department of Corp Science, University of Benin, were bought from a local market in Benin City, Edo State, Nigeria. Seed viability was assessed by floatation method. The seeds were placed in a beaker containing tap water and stirred. The seeds that did not float were regarded as viable seeds.

Crude oil and fractionation: Bonny Light crude oil [^{o}API (American Petroleum Institute) gravity =37] was obtained from Warri Refinery and Petrochemical Company, Delta State, Nigeria. A portion of the crude oil was fractionated by the method of (9) into water soluble fraction (WSF) and water insoluble fraction (WIF). For the fractionation, a 1:2 dilution of 200ml of crude oil was put in a 1 litre conical flask and constantly stirred with a magnetic stirrer for 48h. The WSF then separated from the WIF in a separating funnel.

Assay Methods

Determination of Chlorophyll Content: The chlorophyll content of the fresh leaves of maize in the various treatments was determined by a colorimetric method described by (10). In this method, the leaves (0.5g) were ground in 10ml 80% acetone in the dark and centrifuged at 3,000 g for 5 minutes. The absorbance of the recovered supernatant was read at 645 and 663 nm. Values of optical densities (ODs) were used to calculate total chlorophyll contents using the formula:

Total chlorophyll (mg g^{-1} fw) = $[(20.2(OD_{645}) + 8.02(OD_{663})]/(D \times 1000 \times W) \times V$

where, fw is fresh weight, D is distance travelled by the light path; W is weight of the leaf material taken; V is volume of the extract; OD is optical density.

Determination of starch and glucose: Starch determination was carried out after enzymatic hydrolysis into glucose. Starch and glucose were estimated by the RANDOX test kit (Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY), according to the manufacturer's instructions. Bio-molecular activities, based on enzyme-linked formation of NADPH, were expressed as a specific activity measured at 570 nm. The corresponding concentration was determined against hydrogen peroxide standard curve prepared by using hydrogen peroxide solution. The amount of sugar was expressed as mg g^{-1} fresh weight and all experiments were performed in triplicate.

Statistical Analysis: The result of the study was expressed as mean ± standard error of mean (SEM). Analysis of variance was used to test for differences in the groups while Duncan's multiple comparisons test was used to determine significant differences between means. The Instat-Graphpad software, San Diego, California, USA, was used for this analysis. A $P<.01$ was considered statistically significant.

Results

The results of total chlorophyll content for maize leaves reduced significantly for all fractions and at all levels of contamination when compared with control. The reduction was dose dependent, with the least chlorophyll content observed in the WIF (Table 2). This result is in agreement with the report of (11, 12 and 13) who observed inhibited chlorophyll content of *Corchorus olitorius* in oil polluted soil, low chlorophyll level in *Rhizophora mangle* in oil polluted soil and no irradiation-induced increase in chlorophyll in Wheat (*Triticum aestivum* L.) respectively. It is however at variance with the result of (14) who reported 64.5% increment in total chlorophyll in two wheat genotypes seedlings that were irradiated at 100 gamma radiations but later observed gradual decrease at a higher (200) gamma radiation dose.

The results of the starch content of the roots of the plant showed significant ($P < 0.05$) reduction with plant age being maximum (10.28 mg g^{-1} fresh weight) at 2% of WSF of crude oil contamination 7 days post germination (DPG) while it was minimum (4.85 mg g^{-1} fresh weight) at 10% of WIF of crude oil contamination 21 DPG when compared with the control (Table 3). This result is in agreement with other reports that, total carbohydrates were, decreased with application of zinc in *Salvia officinalis* L. plants (15 and 16).

The results of the glucose content of the roots of the plant significantly increased ($P < 0.05$) in the study when compared with the control. The WSF recording the highest glucose content in the root of the *Z. mays* seedlings. Earlier report on increase in glucose content was also recorded on ionizing radiation (17 and 18).

Table 1: Concentration of crude oil contamination in soil

Group	% Contamination	Number of bags
Control	0%	30
2% Whole crude (WC)	2%	30
5% WC	5%	30
10% WC	10%	30
2% Water soluble fraction (WSF)	2%	30
5% WSF	5%	30
10% WSF	10%	30
2% Water insoluble fraction (WIF)	2%	30
5% WIF	5%	30
10% WIF	10%	30

Table 2. Variation in the total chlorophyll (mg g^{-1} fw) of *Zea mays* exposed to different fractions of crude oil

2% Contamination				
Days /Sample	Control	WC	WSF	WIF
7	8.02±0.04^{a}	3.19±0.09^{b} (39.78%)	3.68 ±0.03^{c} (45.89%)	3.02±0.03^{b} (37.66%)
14	8.21±0.05^{a}	4.07 ±0.03^{b} (49.57%)	4.24±0.02^{c} (51.64%)	4.00±0.02^{b} (48.72%)
21	8.74±0.05^{a}	5.20±0.02^{b} (59.50%)	6.05±0.03^{c} (69.22%)	4.95±0.02^{b} (56.64%)
5% Contamination				
Days /Sample	**Control**	**WC**	**WSF**	**WIF**
7	8.02±0.04^{a}	4.07±0.04^{b} (50.75%)	5.19 ±0.03^{c} (64.71%)	5.62±0.03^{d} (70.08%)
14	8.21±0.05^{a}	6.77 ±0.03^{b} (82.46%)	6.27±0.02^{c} (76.37%)	7.94±0.02^{d} (96.71)
21	8.74±0.05^{a}	8.77±0.02^{a} (0.34%)	6.98±0.02^{c} (79.86%)	9.22±0.02^{d} (5.49%)
10% Contamination				
Days /Sample	**Control**	**WC**	**WSF**	**WIF**
7	8.02±0.04^{a}	5.45±0.03^{b} (67.96%)	6.28 ±0.02^{c} (78.30%)	5.44±0.03^{b} (67.83%)
14	8.21±0.05^{a}	6.10 ±0.03^{b} (74.30%)	6.41±0.03^{c} (78.08%)	6.37±0.02^{c} (77.59%)
21	8.74±0.05^{a}	7.27±0.03^{b} (83.18%)	8.04±0.04^{c} (91.99%)	7.20±0.02^{b} (82.38%)

Values are mean of three (n=3) replicates ± standard error of mean. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIF = Water Insoluble Fraction of crude oil. Values in parenthesis represent percent variation. Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpa

Table 3. Variation in starch content (mg g^{-1} fw) of *Zea mays* exposed to different fractions of crude oil

2% Contamination				
Days /Sample	Control	WC	WSF	WIF
7	13.45±0.03^{a}	9.94±0.05^{b}	10.28±0.02^{c}	8.51±0.14^{d}
14	9.26±0.02^{a}	8.15 ±0.04^{b}	8.47±0.05^{c}	7.19±0.06^{d}
21	7.54±0.04^{a}	6.46±0.03^{b}	6.34±0.17^{c}	6.34±0.05^{d}
5% Contamination				
Days /Sample	**Control**	**WC**	**WSF**	**WIF**
7	13.45±0.03^{a}	8.76±0.05^{b}	9.16 ±0.05^{c}	8.84±0.03^{d}
14	9.26±0.02^{a}	7.32 ±0.02^{b}	7.83±0.03^{c}	7.59±0.04^{d}
21	7.54±0.04^{a}	6.52±0.06^{b}	6.47±0.02^{c}	6.12±0.03^{d}
10% Contamination				
Days /Sample	**Control**	**WC**	**WSF**	**WIF**
7	13.45±0.03^{a}	9.00±0.07^{b}	10.21±0.02^{c}	8.66±0.17^{d}
14	9.26±0.02^{a}	7.98±0.06^{b}	8.36±0.06^{c}	6.46±0.02^{d}
21	7.54±0.04^{a}	6.11±0.03^{b}	6.04±0.06^{b}	4.85±0.05^{d}

Values are mean of three (n=3) replicates ± standard error of mean. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIF = Water Insoluble Fraction of crude oil. Values in parenthesis represent percent variation. Means of the same row carrying different notations are statistically different at P<0.05 using Instat graphpad.

Table 4. Variation in glucose content (mg g^{-1} fw) of *Zea mays* exposed to different fractions of crude oil

2% Contamination				
Days /Sample	Control	WC	WSF	WIF
7	0.05±0.01^{a}	0.06±0.01^{b}	0.05±0.01^{a}	0.06±0.01^{c}

14	0.08±0.01[a]	0.07±0.01[b]	0.07±0.01[b]	0.08±0.01[a]
21	0.06±0.01[a]	0.09±0.01[b]	0.09±0.01[b]	0.09±0.01[b]
5% Contamination				
Days /Sample	**Control**	**WC**	**WSF**	**WIF**
7	0.05±0.01[a]	0.06±0.01[b]	0.07±0.01[c]	0.06±0.01[b]
14	0.08±0.01[a]	0.08±0.01[a]	0.09±0.01[b]	0.08±0.01[a]
21	0.06±0.01[a]	0.10±0.01[b]	0.11±0.01[c]	0.10±0.01[b]
10% Contamination				
Days /Sample	**Control**	**WC**	**WSF**	**WIF**
7	0.05±0.01[a]	0.08±0.01[b]	0.09±0.01[c]	0.08±0.01[b]
14	0.08±0.01[a]	0.10±0.01[b]	0.11±0.01[c]	0.10±0.01[b]
21	0.06±0.01[a]	0.11±0.01[b]	0.11±0.01[b]	0.11±0.01[b]

Values are mean of three (n=3) replicates ± standard error of mean. WC = Whole Crude. WSF = Water Soluble Fraction of crude oil, WIF = Water Insoluble Fraction of crude oil, chlorophyll contents = mg/g wet tissue, Means of the same row carrying different notations are statistically different at $P<0.05$ using Instat graphpad.

Discussion

The significant reduction in chlorophyll content observed in the study may be a result of reduction in the nutrient content of the plant observed earlier by (2). Inadequate supply of nitrogen and magnesium, nutrient elements important for chlorophyll synthesis, may have resulted in the reduction. The hydrophobic nature of the crude oil may have prevented adequate supply of the nutrient elements to the maize plant which invariably may have inhibited chlorophyll synthesis hence the reduction in the chlorophyll content (11 and 12). However, the non significant increase in total chlorophyll content observed in 5% crude oil contamination at 21 days post germination in the root of *Zea mays* may be a consequence of the plant recuperating after exposure.

One of the most studied processes on seedling development is the mobilization of complex polymers such as starch, proteins and lipids from storage tissues such as endosperm or cotyledons. These compounds, considered as seed reserves, are used as energy sources and building blocks for seedling growth during germination (19). The reduction in starch content may have been compensated for by accelerating sugar metabolism and glycolysis (20).

The reduction of starch may indicate that it was the main source of energy in the plant during crude oil exposure which may be related to the fact that starch can be converted into glucose under stress condition as starch (storage carbohydrate) are dissociated as primary metabolites in stress condition to overcome the energy demand by the plant for growth. The reduction in starch contents may also be related with the toxicity of crude oil because starch helps the plant to get energy for survival at the time of emergency. It is not surprising that starch content dropped because it would arise from photosynthesis. As the WC and its fractions lowered total chlorophyll content, photosynthesis will be lowered. Starch may have been metabolized to raise glucose to the level of being able to manage cellular processes and stress in the growing plant. The results suggested that crude oil may have inhibited the growth of the plant observed in an earlier report (2) possibly because of the decrease in starch content. The high glucose content may indicate high energy demand to overcome the stress of growing as well as crude oil toxicity. The results also suggested that starch could be used by the growing plants to produce glucose as energy source to sustain the metabolic activities occurring.

In conclusion, the results showed dose dependent alteration in the metabolic and biochemical processes of the maize seedlings with the water insoluble fraction presenting the most detrimental effect.

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