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Effect of crude oil and simulated acid rain on the growth and physiology of *Thaumatococcus daniellii*

I.A. Ekpo [1,2*], R.B. Agbor [1], E.C. Okpako[1], A. N. Osuagwu[1], B.E Ekanem[1,3], P.A. Otu[1]

[1]*Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross River State, Nigeria.*

[2]*Department of Biological Sciences, Federal University Lafia, Lafia, Nasarawa State, Nigeria.*

[3]*Department of Science Technology, Akwa Ibom State, Polytechnic Ikot-Osurua, Nigeria*

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Abstract

This research work was carried out to investigate the effect of crude oil and simulated acid rain (SAR) on the growth and physiology of *Thaumatococcus daniellii*. 24 seedlings were planted on soil with different concentrations of crude oil 0.5ml, 2.5ml, 5.0ml, 7.5ml, 10ml, 12.5ml, 15ml and 17.5ml, while soil without crude oil served as the control (0ml). On the other hand, simulated acid rain (SAR) was prepared in varying pH values 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, and 5.4 while pH 7.0 served as the control. Results showed that there were no significant differences ($p>0.05$) in the morphological and physiological traits of plants treated with crude oil at all concentrations except in the percentage dry matter content which showed significance at $p<0.05$. Plants treated with simulated acid rain (SAR) showed significant differences ($p<0.001$) in petiole length, plant height, SO_4^- accumulation, Al^{3+}, H^+ accumulation and percentage dry matter content. Results indicated that *Thaumatococcus daniellii* can be exploited in phytoremediation. Also, there is an indication that the plant species cannot thrive favourably on high acidic soil.

***Corresponding Author:** I.A. Ekpo ✉ agborreagan@yahoo.com

Introduction

Thaumatococcus daniellii commonly known as sweet prayers plant, miracle berry, Katemfe, Yoruba soft cane, African serendipity berry, is a species of tropical flowering plants known for being the natural source of thaumatin, mixture of extremely sweet protein which is of interest in the development of sweeteners (Onwueme *et al*., 1979). *T. daniellii* is a large rhizomatous flowering herb native to the rainforests of Ghana and the surrounding African Nations particularly in Southern parts of Ghana, Cote d'ivoire and Nigeria. *T.daniellii* are of great economic importance ranging from cultivation as Fettish plant in Gabon (Mansfeld 1986) to collecting leaves from wrapping and boiling of food in Ghana and Nigeria. The fruits are also used as a low calories taste /flavor modifiers with commercial potential. The petioles are used in weaving mats and baskets (Wiersema and Leon, 1999, Onwueme *et al*., 1979).

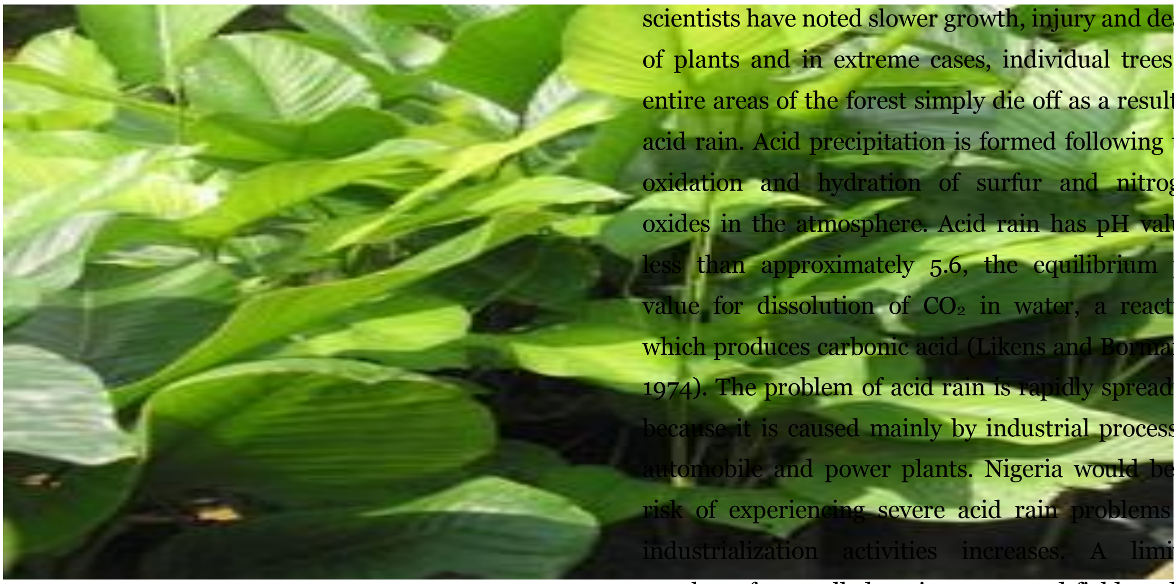

Fig. 1. *Thaumacoccus daniellii*

Crude oil pollution has been a major concern in Nigeria and in the world as it leads to reduction of agricultural productivity and abandonment of farmlands by farmers. A lot of remediation methods have been applied but are very expensive to carry out example, using engineered microbes (e.g Superbug). Fallows from crude oil pollution of soils are known to have undesirable effect on plant growth. Oil spillage constitutes by far the most significant source of pollution and has damaging effect on the entire ecosystem due to the reduction in the level of available plant nutrients or rise in the toxic level of certain elements (Dejong, 1980) as a result of the presence of a significantly high amount of polycyclic aromatic hydrocarbon compounds (Fetzer, 2002). The use of plants for phytoremediation of petroleum contaminated soils is an emerging technology which is cheaper and effective. Certain plant example *Vicia faba* and *Zea mays* are capable of growing on soils polluted with hydrocarbons. These plants degrade through the rhizosphere, part of the root which favours the growth of several microorganisms (Quinones-aquila *et al*., 2003). *Thaumatococcus daniellii* has the ability to grow on various soil types and can regenerate easily even after bush burning (Simon and Sinclair, 2004).

Acid precipitation has been identified as a major environmental/global concern. Over the years, scientists have noted slower growth, injury and death of plants and in extreme cases, individual trees or entire areas of the forest simply die off as a result of acid rain. Acid precipitation is formed following the oxidation and hydration of surfur and nitrogen oxides in the atmosphere. Acid rain has pH values less than approximately 5.6, the equilibrium pH value, for dissolution of CO_2 in water, a reaction which produces carbonic acid (Likens and Bormann, 1974). The problem of acid rain is rapidly spreading because it is caused mainly by industrial processes, automobile and power plants. Nigeria would be at risk of experiencing severe acid rain problems as industrialization activities increases. A limited number of controlled environment and field studies of crop species exposed to simulated acid rain treatments have been conducted. However, before now there was no report on the effect of crude oil and stimulated acid rain on the growth performance of *Thaumacoccus daniellii*.

Materials and methods

Seedlings of *Thaumatococcus daniellii* were obtained from the humid forest vegetation of Akpabuyo Local Government Area in Cross River

State, Nigeria. 54 planting bags were used for the study. The bags were perforated to allow for proper aeration and drainage.

Crude oil preparations

Crude oil was obtained from the Department of Petroleum Resources of the Nigeria National Petroleum Corporation (NNPC). Different concentrations of crude oil were used viz: 0.5ml, 2.5ml, 5.0ml, 7.5ml, 10.0ml, 12.5ml, 15.0ml and 17.5ml. Treatment was applied alternatively with deionized water at an interval of 2 days for 4 weeks. Soil without crude oil served as the control. The experiment was repeated 3 times.

Simulated acid rain

I molar solution each of sulphuric acid (H_2SO_4), nitric acid (HNO_3) and sodium hydroxide (NaOH) were prepared into varying pH values ranging from 4.0, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2 and 5.4, pH (7.0) served as the control. 100ml of the simulated acid rain (SAR) at each pH range were applied to 24 bags containing seedlings. Treatment was applied at 2 days interval for a period of 4 weeks. The experiment was repeated 3 times.

Chlorophyll analysis

Fresh leaves were harvested after four weeks from each stand, ground in a porcelain mortar and extracted in 80% acetone using 1g of sample in 4ml of acetone and analysed using spectrophotometer at 645nm and 663 wavelength (Arnold *et al.*, 1986).

Data analysis

The complete randomized design (CRD) was used with 3 replicates. Data were collected based on the following parameters: leaf area, petiole length, plant height, number of leaves, chlorophyll content, sulfate ion, aluminum oxide, hydrogen ion and percentage dry matter content. Data collected were descriptively analysed and subjected to analysis of variance (ANOVA) test and significant means were separated using least significant different test (LSD).

Results and Discussion

The general comparability of control and crude oil treatment means on *Thaumatoccus danielli* suggest that crude oil treatments did not affect leaf area, petiole length, plant height, number of leaves, chlorophyll content, sulfate ion accumulation and hydrogen ion accumulation. Significant difference at $p < 0.05$ on the percentage dry matter content was however apparent (Table 1 and Table 2). The dry matter content of the control was not significantly different from 0.5, 2.5, 5.0, 7.5, 10.0 and 12.5ml of crude oil treatments. Similar results have been recorded by Njoku *et al.,* (2008). They demonstrated that *Glycine max* germinates and grows in crude oil polluted soil. Frick *et al.,* (1999) included *Glycine max* in the list of plants that can grow and remediate petroleum hydrocarbon contaminated sites. Although, crude oil treatment means of leaf area, petiole length, plant height, number of leaves, chlorophyll content were lower than the control, the differences were not statistically significant suggesting that any inhibitory effect of crude oil were transitory and were not very important to the final yield.

Simulated acid rain soaked soils produced plants with significantly different ($p<0.001$) petiole length, plant heights, sulfate ion accumulation, aluminum ion accumulation as well as percentage dry matter content. However, significant difference ($p>0.05$) were not found in the leaf area, number of leaves and chlorophyll content. Relative to the control, petiole length at all pH level were significantly lower. Results indicated that the more acidic the pH, the shorter the petiole length. Plant heights were significantly ($p< 0.001$) reduced by simulated acid rain treatments relative to the control at all pH levels. Plants treated with pH 5.4, 5.2, 5.0 and 4,8 produced height that were not significantly different while pH 4.0 and 4.2 produced the shortest plants. The leaf number in comparison to control did not show significant difference statistically. Relative to control, the chlorophyll content in all the acid raifn treatments were lower but these differences were not statistically significant and did not affect the final yield. Similar results were recorded for *Capsicum annum*, *Hycopersicon esculentum* and *Solanum melongena* by Verma *et al*., (2010). They reported that chlorophyll was more or less affected following treatment with acid rain at pH 3.0, 4.0 and 5.0. Sulfur ion accumulation was significantly higher in all the acid rain treatments relative to the control.

Aluminium ion accumulated more in the acid rain treated plants relative to the control. According to Sharma and Kair, (1994) toxicity of aluminum may be blocking nutrient uptake which is reflected in the leaves with decreased chlorophyll content. Hydrogen ion accumulation in comparison with the control was increased significantly in the plants treated with acid rain. Percentage dry matter content were significantly lower than the control at all pH levels of the treatment except at pH 5.2 which was not significantly different (p>0.05) from the control. These suggest active shoot growth reduction in these treatments. The general comparability of control and acid rain treatment at highly acidic pH levels (4.0-4.8 adversely affect) *T.daniellii* while pH levels between 5.2-5.6 supports the growth of *T. danielllii*.

Table 1. Data for morphological and physiological characteristics of T. daniellii grown on acids rain soaked soil.

	Morphological traits					Physiological traits			
	Leaf area	Petiole length	Plant height	Number of leaves	Chlorophyll content	SO_4^-	Al^{3+}	H^+	% dry matter content
pH4.0	250.0^{a}±17.72	13.9^{a}±3.18	25.1^{a}±2.57	3.7^{a}±0.58	2.2^{a}±0.33	0.2^{b}±0.01	0.3^{b}±0.01	0.4^{e}±0.01	75.7^{b}±4.16
pH4.2	238.9^{a}±37.22	14.0^{a}±2.78	23.3^{a}±2.25	4.7^{a}±2.31	2.2^{a}±0.32	0.2^{b}±0.01	0.3^{c}±0.01	0.4^{e}±0.01	57.6^{a}±9.62
pH4.4	264.1^{a}±22.47	17.5^{a}±1.46	26.7^{b}±1.37	4.7^{a}±0.58	2.5^{a}±0.22	0.2^{b}±0.01	0.3^{b}±0.01	0.4^{e}±0.01	75.1^{b}±6.38
pH4.6	246.3^{a}±30.13	19.7^{b}±4.18	28.9^{b}±2.98	4.0^{a}±1.00	2.3^{a}±0.18	0.3^{b}±0.03	0.3^{c}±0.01	0.35^{d}±0.01	79.8^{b}±7.79
pH4.8	244.2^{a}±37.27	19.0^{b}±2.65	30.1^{c}±2.27	3.3^{a}±1.15	2.4^{a}±0.27	0.3^{b}±0.02	0.3^{b}±0.01	0.34^{d}±0.01	78.0^{b}±1.64
pH5.0	276.4^{a}±25.26	20.2^{b}±2.00	30.7^{c}±1.50	4.0^{a}±1.00	2.2^{a}±0.05	0.3^{b}±0.01	0.3^{b}±0.01	0.29^{c}±0.01	83.6^{b}±1.59
pH5.2	213.6^{a}±53.45	21.1^{b}±1.28	31.0^{c}±1.29	3.7^{a}±1.53	2.4^{a}±0.55	0.3^{c}±0.01	0.3^{b}±0.03	0.28^{c}±0.01	91.6^{c}±4.89
pH5.4	232.3^{a}±13.56	22.1^{b}±1.72	32.1^{c}±1.30	3.3^{a}±0.58	2.4^{a}±0.33	0.3^{c}±0.01	0.3^{b}±0.01	0.23^{b}±0.01	77.6^{b}±5.15
pH7.0	300.5^{a}±59.53	27.6^{c}±1.95	34.1^{d}±0.90	4.7^{a}±0.58	2.8^{a}±0.06	0.2^{a}±0.01	0.2^{a}±0.01	0.18^{a}±0.01	92.5^{c}±6.05
LSD	NS	4.31	3.32	NS	NS	0.024	0.0204	0.013	9.96

Data were expressed in mean and standard error (X ±S.E) in triplicate. *Means followed with the same case letter along vertical array shows no significant difference ($p < 0.05$).

Table 2. Data for morphological and physiological characteristics of *T. daniellii* grown on crude oil polluted soil.

Morphological traits					Physiological traits				
	Leaf area	Petiole length	Plant height	Number of leaves	Chlorophyll content	SO_4^-	Al^{3+}	H^+	% dry matter content
pH4.0	250.0^{a}±17.72	13.9^{a}±3.18	25.1^{a}±2.57	3.7^{a}±0.58	2.2^{a}±0.33	0.2^{b}±0.01	0.3^{b}±0.01	0.4^{e}±0.01	75.7^{b}±4.16
pH4.2	238.9^{a}±37.22	14.0^{a}±2.78	23.3^{a}±2.25	4.7^{a}±2.31	2.2^{a}±0.32	0.2^{b}±0.01	0.3^{c}±0.01	0.4^{e}±0.01	57.6^{a}±9.62
pH4.4	264.1^{a}±22.47	17.5^{a}±1.46	26.7^{b}±1.37	4.7^{a}±0.58	2.5^{a}±0.22	0.2^{b}±0.01	0.3^{b}±0.01	0.4^{e}±0.01	75.1^{b}±6.38
pH4.6	246.3^{a}±30.13	19.7^{b}±4.18	28.9^{b}±2.98	4.0^{a}±1.00	2.3^{a}±0.18	0.3^{b}±0.03	0.3^{c}±0.01	0.35^{d}±0.01	79.8^{b}±7.79
pH4.8	244.2^{a}±37.27	19.0^{b}±2.65	30.1^{c}±2.27	3.3^{a}±1.15	2.4^{a}±0.27	0.3^{b}±0.02	0.3^{b}±0.01	0.34^{d}±0.01	78.0^{b}±1.64
pH5.0	276.4^{a}±25.26	20.2^{b}±2.00	30.7^{c}±1.50	4.0^{a}±1.00	2.2^{a}±0.05	0.3^{b}±0.01	0.3^{b}±0.01	0.29^{c}±0.01	83.6^{b}±1.59
pH5.2	213.6^{a}±53.45	21.1^{b}±1.28	31.0^{c}±1.29	3.7^{a}±1.53	2.4^{a}±0.55	0.3^{c}±0.01	0.3^{b}±0.03	0.28^{c}±0.01	91.6^{c}±4.89
pH5.4	232.3^{a}±13.56	22.1^{b}±1.72	32.1^{c}±1.30	3.3^{a}±0.58	2.4^{a}±0.33	0.3^{c}±0.01	0.3^{b}±0.01	0.23^{b}±0.01	77.6^{b}±5.15
pH7.0	300.5^{a}±59.53	27.6^{c}±1.95	34.1^{d}±0.90	4.7^{a}±0.58	2.8^{a}±0.06	0.2^{a}±0.01	0.2^{a}±0.01	0.18^{a}±0.01	92.5^{c}±6.05
LSD	NS	4.31	3.32	NS	NS	0.024	0.0204	0.013	9.96

Conclusion

Crude oil remediation/ sequestration as well as acid rain tolerance by plants is vital in environmental protection and food security in Nigeria. The non significant effects recorded in the morphological and physiological characteristics of *T.daniellii* grown on crude oil polluted soil suggest that *T.daniellii* can thrive on oil spill environments and can be considered for further research on phytoremediation. On the other hand, significant effects recorded for plants treated with simulated acid rain indicates that *T.daniellii* cannot withstand/ tolerate very high acidic pH level of liquid.

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