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Evaluation on the growth response of Peanut (*Arachis hypogaea*) and Sorghum (*Sorghum bicolor*) to crude oil contaminated soil

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ABSTRACT: This study examined the cytotoxic response, germination, survival, morphological deviations as well as enzyme activities of *Arachis hypogaea* and *Sorghum bicolor* in crude oil contaminated soil. Crude oil spillage was simulated to achieve 1%, 2%, 3% (w/w) contamination levels in pot experiments. Treatments without crude oil were used as control. Cytotoxicity, germination and survival were determined by using percentages while enzyme activity was measured by using spectrophotometric methods and standard curves. *S. bicolor* had lower mitotic index (3.7) with higher percentage aberrations (65.56%) compared to *A. hypogaea*. However, difference in mitotic index and percentage aberration between *A. hypogaea* and *S. bicolor* was not significant at $P \geq 0.05$. Percentage germination and survival of both plants were not different. Enzyme study showed that enzyme activity in *A. hypogaea* and *S bicolor* were the same in control but increased with crude oil contamination. Laccase activity was significantly higher ($P \leq 0.05$) in *A. hypogaea* than in *S. bicolor* in 1% and 2% crude oil concentrations. The same applies to soluble methane monoxygenase activity in all crude oil concentrations. Tyrosinase activity was not significantly different in both plants in all concentrations. The study shows *A. hypogaea* to have better tolerance in crude oil contaminated soils than *S. bicolor*. © JASEM
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Keywords: *S. bicolor*, *A. hypogaea*, crude oil, pollution, response

Nigeria has recorded high occurrence of oil spillage in the Niger Delta (Ordinioha and Brisibe 2013) and along pipelines traversing other parts of the country mainly due to sabotage, corrosion and rupture of pipelines, operations and transportation among others (Adelana *et al*., 2011). According to NOSDRA (2016), oil spillage in Nigeria has escalated to a thousand spills per year, the highest rate of spills worldwide suggesting the need for continuous search of the best methods of cleaning up crude oil to control its negative impacts on the environment. Some of these impacts among others include loss of mangrove ecosystem (Agbogidi *et al*., 2006), loss of agricultural land and poor crop harvest (Obi, 2012).

Crude oil spills affect plants adversely by creating conditions which make essential nutrients like nitrogen and oxygen needed for plant growth unavailable to them. Chromosomal aberrations, morphological changes and reproductive success in plants have been linked to the effects of contaminants in the soil. Using plants to control pollutants (phytoremediation) in the soil has become a widely accepted approach and consequently, plants viability in the zone of contamination is a critical issue in the successful application of phytoremediation. Selection of plants for remediation of soils with organic pollutants have been based on their resistance to pollutant phytotoxicity (Kirk *et al*., 2002), which manifests in their ability to germinate, grow and survive in the polluted medium. It is also based on the presence of enzymes and phenolic compounds in root exudate and plant tissues (Liste and Alexander, 1999) or their capability to reduce pollutant concentration in the soil. More so numerous studies have demonstrated that plant chromosomes are sensitive indicators to environmental pollutants (Grant, 1998). This study therefore examines the potentials of *A. hypogaea* and *S. bicolor* in a crude oil contaminated soil through their physiological and growth response.

The choice of *A. hypogaea* and *S. bicolor* in this study stems from the fact that grasses have multiple ramified root systems that give room for rhizosphere effect while legumes are known for nitrogen fixing which offers them the ability to grow in nitrogen deficient soil of crude oil contaminated zones.

MATERIALS AND METHODS

Samples and sources: The soil used for this study was sandy loam soil from University of Lagos uncultivated rain forests, identified according to the method specified by the British Standard Institution (BSI) for soil tests for civil engineering purposes, BS1337: part 2 (1990). Crude oil was obtained from Exonn Mobil floating production storage and off-loading facility while seeds of *Arachis hypogaea* (NG/SA/JAN/09/154) and *Sorghum bicolor* (NG/TD/APR/09/008) were obtained from the gene bank of National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan Oyo state Nigeria.

Soil preparation and planting: The soil was air-dried and passed through a 5mm sieve to remove stones and debris for easy absorption of crude oil (Njoku *et al*., 2012). Conditions of oil spill were simulated by adding different amounts of crude oil (100g, 200g and 300g) to 10kg soil in experimental pots by spraying and continuous mixing to get 1%, 2% and 3% levels of contamination. Soil without crude oil contamination (0%) was used as control. Three replicates were made for each treatment. The modified method of Eze *et al*. (2013) was adopted by planting eight seeds of peanut and sorghum in different containers of depth 10cm and circumference of 100cm. The pots were watered every alternate day.

Methodology: Germination of seeds was scored within 10 days of planting starting from the 3rd day. The yardstick for germination was the appearance of the plumule for sorghum and the radical for peanut. Percentage germination and survival of the seedlings of the plants in crude oil contaminated soil was calculated as was described by Njoku (2008). The mitotic index and chromosomal aberrations were evaluated using the methods of Odeigah *et al*. (1997) and Incer *et al*. (2003). The protein content and enzyme activities of the plant tissues were determined by the methods described by Garcia-Monila *et al.* (2007).

Statistical analyses: Statistical differences among the growth responses of the two plants were determined by using chi square test and student's t- test. Difference was considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Germination of *A. hypogaea* and *S. bicolor*: There was a 100% germination of the seeds of both *A. hypogaea* and *S. bicolor* in the soil without crude oil while lower percentage germination occurred in soils with different crude oil concentrations. The more the crude-oil contamination the lower the germination percentage for both *A. hypogaea* and *S bicolor*, with 3% crude oil contaminated soil showing the least percentage germination. However, there was no significant difference ($P>0.05$) in the germination performance of *A. hypogaea* and. *S bicolor* in all crude oil contamination levels (table 1). The uniformity in germination of *A. hypogaea* and *S. bicolor* may imply that both plants have equal tendencies to resist the effect of crude oil. This may be a reason for their existence in the wild in oil contaminated fields of Iran (Shirdam *et al.,* 2008). The progressive decrease in the germination of both plants as crude oil contamination increased corresponds with the reports of earlier studies by Merkl *et al*. (2004b), Njoku (2008) and Oyedeji (2012). The decrease was due to penetration of the oil into the seeds which is believed to have killed the embryos or may act as a physical barrier around seeds thus preventing or reducing both oxygen and water from entering the seed.

Table 1: Germination of *A. hypogaea* and *S. bicolor* in crude oil contaminated soil.

Percentage Concentration of crude oil	*A. hypogaea*		*S. bicolor*		χ^2
	NG	PG (%)	NG	PG (%)	
0%	8.00	100	8.00	100	0.000ns
1%	7.00	83.33	7.00	83.33	0.00ns
2%	6.00	79.33	6.00	79.33	0.00ns
3%	5.00	58.67	5.00	58.67	0.00ns

Values represent mean ± SD (n=3); NG: number of seeds that germinated; PG: percentage seed germination; ns: difference not significant at $P \geq 0.05$

Survival of A. hypogaea and S. bicolor: Percentage survival of *A. hypogaea* was not significantly higher than that of *S. bicolor* in all the crude oil contaminated soils. There was also a decline in mean survival of both plants, with increase in crude oil contamination in the soil (table 2). The slightly higher level of survival of *A. hypogaea* in all the levels of contamination was an indication that it tolerates crude oil contamination more. This was probably caused by the innate resistant qualities of *A. hypogaea* such as nitrogen fixation to the soil and reduction of the C/N ratio thereby reducing the effect of crude oil on its growth or its remarkably lower phytate (an anti-nutrient) content (Eze *et al*., 2013) which facilitates the absorption of essential minerals like calcium, magnesium and phosphorus (Raboy, 2002) for growth.

Table 2: Percentage survival of *A. hypogaea* and *S.bicolor* in crude oil contaminated soil

Crude oil concentration	Percentage survival		
	. hypogaea	*S. bicolor*	$t_{0.05}$
0%	8.00±0.00	8.00±0.00	0.00ns
1%	8.00±0.00	7.33±0.58	2.00ns
2%	6.67±0.58	6.33±0.58	0.71ns
3%	5.67±0.58	4.67±0.58	2.12ns

Values represent mean±SD (n=3); ns: not significant at $P \geq 0.05$

Morphological variations observed in both plants: Stunted growth, chlorosis, leaf burnt and wilting were observed in *A. hypogaea* and *S. bicolor* growing in higher levels (2% and 3%) of crude oil contamination but were more conspicuous in *S. bicolor* than in *A. hypogaea* (plate1). This can be attributed to the low nitrogen content, phosphorus content and high salinity that characterize crude oil contaminated soils (Obasi *et al*., 2013 and Oyem, 2013). According to Oyem (2013), soil salinity causes symptoms such as dead sections of leaf margins, burning and stunted or no growth of plants.

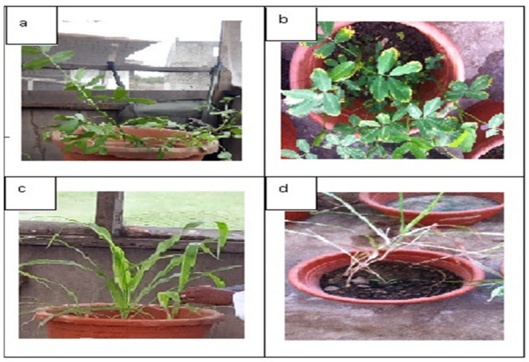

Plate 1: Morphological deviations from normal plants at 6 weeks (a) Control for *A. hypogaea* (b) *A. hypogaea* in 3% crude oil contamination (c) control for *S. bicolor* (e) *S. bicolor* in 3% crude oil contamination.

Cytotoxic response of A. hypogaea and S.bicolor: The cells of the root tips harvested from uncontaminated soil had normal divisions when compared with those from crude oil contaminated soil. Cells of both plants in crude oil contaminated soil had chromosomal aberrations such as c-mitosis, sticky chromosomes, anaphase bridges and vagrant chromosome observed (plate 2). There were decreasing mitotic index and increasing percentage aberrations in both plants when exposed to more crude oil contamination. *S. bicolor* had the lower mitotic index (3.7) and the higher percentage aberration (67.56%) at 3% crude oil contamination. The mitotic index of *A. hypogaea* was higher than that of *S. bicolor* in all the crude oil contamination levels except in control. However there was no significant difference (P>0.05) between the mitotic index and percentage aberrations in *A. hypogaea* and that of *S. bicolor* in all the crude oil contamination levels (Figure 1). Chromosomal aberrations and decrease in the mitotic index of *A.hypogaea* and *S. bicolor* observed in this study is similar to the results obtained by Akinola and Njoku (2007) and Komolafe *et al*. (2015). The decrease in the mitotic indices of the cells of both plants shows that crude oil contamination inhibits cell division in the roots of plants thereby hindering growth and development. The chromosomal aberrations seen in this study was also in line with the reports of Njoku *et al.* (2011) who posited that exposure of plants to higher concentrations of crude oil may alter chromosome structure and arrangement during meiosis and possibly affect survival and existence of the plants in crude oil contaminated soil. The higher percentage aberration and the lower mitotic index shown by *S. bicolor* is an indication that it is more sensitive to the effect of crude oil than *A. hypogaea* and this may have accounted for its lower survival rate than *A. hypogaea.* This is corroborated by the findings of studies on the effect of other forms of petroleum. For instance Ogbo (2009) reported more adverse effects on *S. bicolor* than *A. hypogaea* exposed to diesel oil contamination.

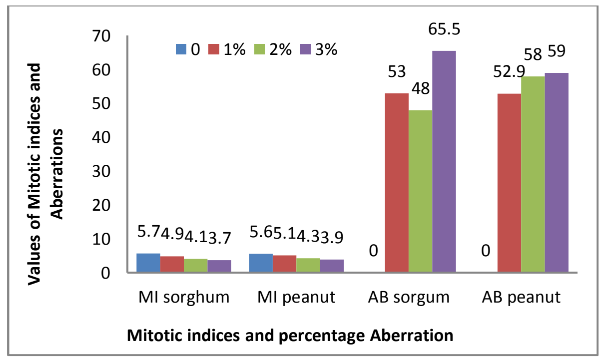

Fig 1: Mitotic index and percentage aberrations in *A. hypogaea* and *S. bicolor* in varied crude oil concentrations. Legend: MI: Mitotic Index, AB: Percentage Aberrations,

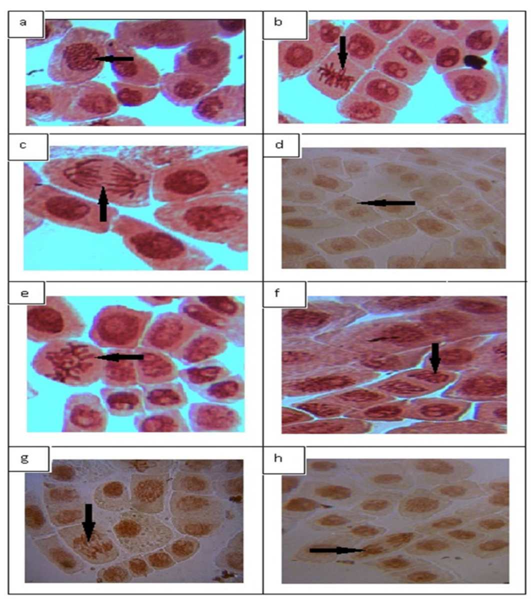

Plate 2: Observed cell activities in varied crude oil contaminated treatments (a-d) Normal mitotic division (e-h) aberration. Legend: The arrows are pointing at (a) Prophase (b) metaphase (c) anaphase (d) telophase (e) c-mitosis (f) sticky cell (g) anaphase bridge (h) vagrant.

*Enzyme activities of A. hypogaea and S. bicolor***:** Soluble methane monoxygenase had the highest activity in both plants followed by laccase and tyrosinase. Laccase and soluble methane monoxygenase activities in *A. hypogaea* were significantly higher (P≤0.05) than in *S. bicolor* except in 3% contamination where there was no significant difference (P≥ 0.05) in laccase activity of both plants. There was no significant difference (P≥ 0.05) between the activities of tyrosinase in both plants (table3). Higher activities of soluble methane monoxygenase and laccase enzymes observed in *A. hypogaea* tissues depict that these enzymes may have been present in larger quantities in its tissues and root exudates. This can be associated to better survival and consequently better growth of *A. hypogaea.* It could also be that *A. hypogaea* habours endophytic bacteria found to express *alkB* genes for production of alkane monoxygenase as was reported to have been found in some legumes such as *Lotus corniculatus* (Andria, 2008). Alkane monoxygenase enzymes contribute to detoxificaton of organic contaminants and better survival of plant under toxic conditions. Wolfe and Hoehamer (2003) reported that laccases and peroxidises released from plant roots transform pollutants into compounds easily absorbed by plants or degradable by rhizosphere microorganisms. Laccases are capable of oxidising many aromatic compounds using dioxygen as the terminal electron acceptor forming an oxidised aromatic product and two molecules of water. The ability to transform recalcitrant aromatic components of crude oil in the soil growing *A. hypogaea* by laccase enzymes may have reduced the effect of crude oil contamination on it.

Table 3: Enzyme activities in *A. hypogaea* and *S. bicolor* in crude oil contaminated soil

Enzyme	Crude oil Concentration	*A. hypogaea*	*S. bicolor*	*t*
Laccase	Control	4.19±1.24	2.88±1.10	1.37
	1%	10.46±0.71	7.34±1.34	3.57*
	2%	15.05±1.23	9.11±1.30	5.76**
	3%	17.67±1.16	15.05±1.77	1.90
Soluble methane	Control	1.31±0.20	1.14±0.18	1.07
Monoxygenase	1%	3.17±0.39	1.84±0.19	5.31*
	2%	3.67±0.31	2.14±0.22	7.02**
	3%	4.49±0.46	3.04±0.35	4.33*
Tyrosinase	Control	0.12±0.07	0.10±0.70	0.40
	1%	0.32±0.14	0.25±0.10	0.74
	2%	0.28±0.20	0.33±0.11	0.38
	3%	0.55±0.16	0.44±0.09	1.03

Values represent mean± Std. dev (n = 3); *P < 0.05; **P < 0.01

*Conclusion***:** The result of this study shows that *A. hypogaea* could tolerate and have the potentials to remediate crude oil contaminated soil better than *S. bicolor* while the latter could do better for bio monitoring of crude oil in the environment.

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