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Mutagenic Effect of Crude Oil on Accessions of *Glycine max* L. (Merril)

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Abstract. Study of the effects of crude oil on four accessions of *Glycine max* showed that the rate of germination, root length development and rate of cell division of the accessions decreased with increasing concentration of crude oil. However, the extent of effects on the accessions varied showing differences in the abilities of the accessions to survive in crude oil polluted sites, the tolerance being in the order of TGX1019-1E < TGX1805-31F < TGX1440-1E < TGX1448-2E suggesting TGX 1019-1E to be the best indicator of and TGX1448-2E to be the best tolerant accession to the crude oil pollution.

Keywords: *Glycine max*, mitotic index, pollution, crude oil pollution, mutagenic effect of crude oil

Introduction

Many researchers have reported adverse effects of crude oil on plants and the ecosystems. Mackin (1950a; 1950b) reported that crude oil causes death of saltgrass and saltwort. Other antagoinstic effects on plant growth reported include inhibition of germination, stunted growth, brown leaves and pale stems (Udo and Fayemi, 1975), reduction and blocking of gaseous exchange in seeds (FEPA, 1990), prevention of water and nutrient uptake by seeds thereby reducing germination and subsequent growth of plants (Adesiyan and Osuji, 1993), reduction in the number and distortion of stomata, morphological and anatomical aberrations (Cole, 1994; Holmer and Bale, 1987). Kinako (1981) found that crude oil pollution leads to reduction of number of plant species within the range of 67-92% with the same trend in productivity and that crude oil tends to cause a drastic slow-down in vegetation recolonization.

Like other contaminants, the effects of crude oil on the biological systems can be studied using test systems or assays, based on plants, mammalia, bacteria, drosophila, etc. which can be used to determine genotoxic effects of the contaminants. Plant assays have been developed to determine the genetic changes induced by contaminants, their metabolites and residues (Veleminsky and Gichner, 1988), based on laboratory, greenhouse or field studies (Ma and Harris, 1985; Plewa, 1985; Grant, 1982). Grant and Zura (1982) and Constantin (1982) reported that several plant assay systems have been used for monitoring genotoxic substances in the environment and assessing the risks to humans. Odeigah *et al.* (1997a; 1997b) used *Allium* test to evaluate the genotoxic effects of waste water and leachate from solid industrial wastes. Most plant assays are usually based on macroscopic studies, wherein the morphological features like growth rate, leaf areas and leaf colouration are used to ascertain the effects of chemicals on plants and microscopic studies using chromosomal aberrations for the same purpose.

Although the importance of using plant assays for determining the effects of contaminants on biological systems has been reviewed by many researchers (Fiskesjo, 1997; Wang, 1992; Sandhu *et al.*, 1991; Kihlman, 1966; Levan, 1951), only few researches used *Glycine max* in the study. The aim of this study is, therefore, to determine the effects of crude oil on *G. max* so as to evaluate its use as a biomonitor of crude oil pollution.

Materials and Methods

Sources of crude oil and *Glycine max*. The crude oil (wellhead medium) used in this study was obtained from Shell Petroleum Development Company, Port Harcourt, Nigeria. The seeds of the four accessions of *G. max* (TGX 1805-31F, TGX 1019-1E, TGX 1440-1E and TGX 1448-2E) were obtained from the Gene-Bank section of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

Planting of seeds. Ten seeds of each accession were planted in a petri-dish lined with a layer of filter paper wetted with a given concentration of crude oil-water mixture. The concentration used were 1%, 5%, 10%, 15%, 20% and 25%, with each serving as a treatment and replicated thrice. For control treatment, the filter paper was kept wet with distilled water.

Germination experiment. The number of seedlings that emerged from each petri-dish four days after planting was counted and used to determine the rate of germination. The protrusion of radicle was used as yardstick for germination. The number of seeds that germinated from each treatment for each accession was summed and the mean germination percentage for each treatment was calculated.

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Root length measurement. The root length of one seedling from each petri-dish was measured five days after germination using a calibrated ruler. The mean root length for each treatment was divided by that of the control and percentage of the quotient was calculated. The percentages were plotted against the treatments and the EC_{50} was calculated for each accession (Odeigah *et al.*, 1997b).

Chromosome analyses. The root tips of the germinating seeds from different treatments were cut and fixed in 3:1 acid alcohol for 24 h. The root tips were then rinsed with distilled water and put in 1N HCl for 5 min. (to soften the tissue), macerated and stained with aceto-orcein for 15 min. The macerated stained roots were then squashed and viewed microscopically to see the dividing cells. The mitotic index of each accession for each treatment was calculated by dividing the number of dividing cells by 1000 and determining the percentage (Inceer *et al.*, 2003).

Analyses of results. The results obtained for germination and root length measurements were statistically analysed using ANOVA and LSD, at 5% probability levels

Results and Discussion

The percentage germination of different accessions of *G. max* in different concentrations of crude oil is shown in Table 1. The highest percentage germination was produced by 1% treatment in all the accessions while 25% treatment produced the poorest percentage germination in all the accessions except for TGX 1448-2E, whereas, 20% treatment produced the poorest percentage germination. The percentage germination in all accessions decreased as the concentration of the crude oil increased except for 1% treatment that produced greater percentage than the control and 15% treatment for TGX1440-2E that has higher percentage germination than 10% treatment. LSD ($P<0.05$) showed that apart from 1% and 5%, all the other treatments were significantly different from the control.

Table 2 shows the effect of crude oil on the root length development of the four accessions of *G. max*. There is a general inverse relationship between the concentrations and the root lengths in all the accessions except for TGX1805-31F (at 1% and 5%), TGX1019-1E (at 1% and 20%) and TGX1448-2E (at 10%). TGX1448-2E produced the highest mean root length of 13.5±1.70 at 1% treatment, TGX1440-1E produced the longest mean root length in all treatments except at 10%. Apart from 1% treatment, all treatments were significantly different from the control (LSD ($P<0.05$). The ratio of the root length for each treatment to that of the control is shown in Table 3. The table shows that the highest ratio accrued to TGX1805-13F (at 1% treatment) followed by TGX1448-2E (at 1%). The EC_{50} for different accessions shows that TGX1019-1E has the lowest EC_{50} (0.8%) while TGX1448-2E has the highest EC_{50} (about 14%) (Fig. 1).

Table 1. Effect of crude oil on the percentage germination of four accessions of *Glycine max* (values ± standard error)

	TGX 1019-1E	TGX1805-31F	TGX 1440-1E	TGX1448-2E
Control	16.67 ± 0.09	33.33 ± 0.22	63.33 ± 0.35	36.67 ± 0.20
1%	33.33 ± 0.18	40.00 ± 0.23	76.67 ± 0.40	56.67 ± 0.30
5%	16.67 ± 0.09	20.00 ± 0.09	56.67 ± 0.31	40.00 ± 0.23
10%	10.00 ± 0.07	16.67 ± 0.13	13.33 ± 0.10	36.67 ± 0.21
15%	3.33 ± 0.03	6.67 ± 0.04	23.33 ± 0.13	16.67 ± 0.13
20%	3.33 ± 0.03	6.67 ± 0.06	13.33 ± 0.13	0.00 ± 0.00
25%	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 0.04

Table 2. Mean root length (cm) of four accessions of *Glycine max* in different concentrations of crude oil

	TGX 1019-1E	TGX1805-31F	TGX 1440-1E	TGX1448-2E
Control	6.02 ± 0.24	6.67 ± 0.36	15.17 ± 0.54	10.00 ± 0.24
1%	2.77 ± 1.70	10.1 ± 0.86	14.00 ± 0.62	13.50 ± 1.70
5%	1.00 ± 0.81	0.50 ± 0.41	8.67 ± 2.34	12.07 ± 1.41
10%	0.23 ± 0.91	1.07 ± 0.87	4.43 ± 0.94	8.43 ± 2.17
15%	0.20 ± 0.16	0.53 ± 0.43	3.80 ± 1.30	3.73 ± 2.57
20%	1.03 ± 0.84	0.60 ± 0.49	2.07 ± 1.69	NG
25%	NG	NG	NG	0.80 ± 0.38

NG = no germination

Table 3. Root length of accessions in different concentrations of crude oil compared with the control

	TGX 1019-1E	TGX 1805-31F	TGX 1440-1E	TGX 1448-2E
Control	100	100	100	100
1%	46.01	151.4	92.29	135.00
5%	16.61	7.50	57.15	120.7
10%	3.82	16.04	29.20	84.3
15%	3.32	7.95	25.05	37.3
20%	17.11	9.00	13.65	0.00
25%	0.00	0.00	0.00	8.00

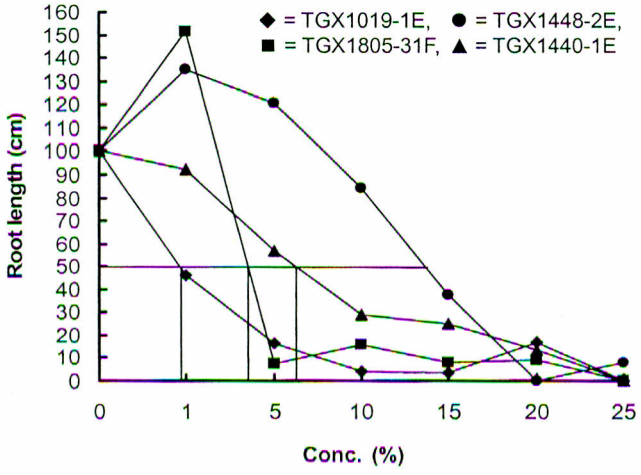

Fig. 1. EC_{50} of different accessions of *Glycine max* exposed to crude oil.

The mitotic stages produced by different accessions in different treatments are shown in Table 4, while the mitotic indices of the accessions in different treatments are shown in Table 5. The mitotic index in each accession decreased as the concentration of crude oil increased. However, deviations from the above trend were noticed in some cases like TGX1805-31F (5%, 10%, 15%, 20% concentrations), TGX1019-1E (15% and 25% concentrations) and TGX1448-2E (20% and 25% concentrations).

The effect of crude oil on the germination of *G. max*, reported in this study, conforms with those reported in earlier studies by Ghosh and Kumar (2000); Atuanya (1987) and Gill and Sandota (1976). The reduction in the rate of germination could be due to the ability of crude oil to damage germinating seeds or plants (Singh *et al.*, 1985). In addition to the death of plant seeds, crude oil could have caused reduced water imbibition and gaseous exchange in the seeds of *G. max* (FEPA, 1990; Zewar, 1988) which are necessary for seed germination. The reduced rate of germination of *G. max* on exposure to crude oil falls in line with the proposition of Cullie and Blanchet (1958) who reported increase in the phytoxicity of oil with the increase in the quantities applied. The same may be one of the causes of reduced vegetation recolonization, habitat degradation like erosion and shortage of food for the consumers (Kinako, 1981). It should be noted that although germination of the accessions decreased as the concentration of crude oil increased, seeds in 1% crude oil treatment germinated better than the control (0% crude oil). This could be the result of

Table 4. Mitotic stages of accessions of *Glycine max* exposed to crude oil

		Concentration of crude oil						
Accession	Mitotic stage	control	1%	5%	10%	15%	20%	25%
TGX1019-1E	Prophase	-	-	-	-	-	-	-
	Metaphase	-	-	-	-	-	-	√
	Anaphase	√	√	-	-	-	-	√
	Telophase	-	-	-	√	√	-	√
TGX1805-31F	Prophase	-	-	-	√	√	-	-
	Metaphase	√	√	-	√	√		-
	Anaphase	√	√		-	-	-	-
	Telophase	√	-	-	√	√	-	-
TGX1440-1E	Prophase	-	√	-	-	-	-	-
	Metaphase	√	√	√	√	√		-
	Anaphase	-	√	√	-	-	-	-
	Telophase	√	-	√	√	√	-	-
TGX1448-2E	Prophase	-	√	-	√	-	-	-
	Metaphase	√	√	√	√	-	-	-
	Anaphase	√	√	√	-	-	-	-
	Telophase	√	√	√	-	-	-	-

√ = present/observed; - = absent/not observed

Table 5. Effect of crude oil on the mitotic indices of accessions of *Glycine max*

Accession		Concentration of crude oil						
		Control	1%	5%	10%	15%	20%	25%
TGX1019-1E	No. of cells counted	1000	1000	1000	1000	1000	1000	1000
	No. of dividing cells	6	0	0	3	6	0	12
	Mitotic index	0.6	0	0	0.3	0.6	0	1.2
TGX1805-31F	No. of cells counted	1000	1000	1000	1000	1000	1000	1000
	No. of dividing cells	12	12	3	9	0	6	0
	Mitotic index	1.2	1.2	0.3	0.9	0	0.6	0
TGX1440-1E	No. of cells counted	1000	1000	1000	1000	1000	1000	1000
	No. of dividing cells	9	12	18	12	12	6	0
	Mitotic index	0.9	1.2	1.8	1.2	1.2	0.6	0
TGX1448-2E	No. of cells counted	1000	1000	1000	1000	1000	1000	1000
	No. of dividing cells	12	18	12	9	0	0	0
	Mitotic index	1.2	1.8	1.2	0.9	0	0	0

stimulation of growth and nodule development (Akinola *et al.*, 2004; Baker, 1970) or due to release of nutrients from crude oil (Baker, 1970).

The reduced root length recorded in *G. max* resulting from crude oil treatment may be due to reduced metabolism, gaseous exchange and translocation (Baker, 1970). The longer root length of the TGX1805-31F and TGX1448-2E accessions of *G. max* may be due to stimulation of growth at low concentration of crude oil as earlier reported (Akinola *et al.*, 2004; Baker, 1970).

Different EC_{50} values for different accessions of *G. max* suggest their different abilities to withstand crude oil pollution. Generally low EC_{50} values suggest susceptibility of *G. max* to crude oil pollution although Frick *et al.* (1999) listed it as one of the plants, tolerant to crude oil.

Inhibition of mitosis by high concentration of crude oil in the accessions of *G. max* indicates phytotoxic effects of crude oil contributing to reduced growth of *G. max* and low mitotic indices observed in this study.

From the findings of this study, it seems obvious that although the four accessions of *G. max* have different sensitivities to crude oil pollution, TGX 1448-2E has the best tolerance. It can also be inferred that TGX 1019-1E and TGX 1805-31F are more sensitive to crude oil pollution than TGX 1440-1E and TGX 1448-2E. Thus exposure of *G. max* to high concentration of crude oil should be avoided as much as possible as it adversely affects all the accessions of *G. max*. In addition, TGX 1448-2E can be studied further to determine its ability to remediate soils contaminated with crude oil while TGX 1019-1E can be further studied for the effects of crude oil on *G. max*.

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