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Germination, Survival and Growth of Accessions of *Glycine max* L. (Merril) (Soybean) and *Lycopersicon esculentum* L. (Tomato) in Crude Oil Polluted Soil

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Abstract: The germination, survival and growth of four accessions each of *Glycine max* and *Lycopersicon esculentum* in crude oil polluted soil were investigated in this study. The TGX 1440-1E accession of *G. max* performed better than the other accessions of *G. max* while the DT 95/370 accession of *L. esculentum* performed better than the other accessions of *L. esculentum*. The accessions of *L. esculentum* survived more in the crude oil polluted soil than the accessions of *G. max* but the accessions of *G. max* grew faster than those of *L. esculentum*. General analysis of the performances of the accessions of the crops showed that TGX 1440-1E accession of *G. max* performed better than other accessions of *G. max* and *L. esculentum* used in the study. This suggests that intraspecific differences exist in the way plants respond to crude oil pollution. Such intraspecific differences can be utilized in planning for crops to be planted in crude oil polluted soil.

Key words: *Glycine max*, *Lycopersicon esculentum* crude oil, germination, survival, growth

INTRODUCTION

Petroleum exploration, exploitation, processing and transportation lead to the pollution of soil with crude oil, refined and waste products of petroleum (Nicolloti and Eglis, 1998). The polluted soil usually becomes less useful for agricultural and other activities like recreation and the soil organisms and soil dependent organisms are adversely affected (Baker, 1970; Gudin and Syratt, 1975; Mackay, 1991; Gelowitz, 1995; Siddiqui and Adams, 2002; Lundstedt, 2003). The crude oil polluted soil has changed soil properties due to the hydrophobic behaviour of oil which results in the reduction of water and nutrient availability (Baker, 1970; Bossert and Bartha, 1985). Crude oil pollution also leads to high mechanical resistance in the soil which slows down root elongation (Bengough, 2003). Oil contamination can also alter the soil moisture conditions and due to the hydrophobic nature of oil, water spreads inhomogenously in oil-contaminated soils (Merckl *et al*., 2005a, b). This leads to water deficiency is such soils.

Apart from affecting the soil, crude oil affects the growth of plants. According to Merckl *et al.* (2005a, b) crude oil leads to elimination of vegetation cover and subsequent soil erosion. Merckl *et al.* (2004a, b) reported that crude oil pollution reduces the rate of seedling emergence and growth of plants. The work of Agbogidi *et al.* (2006) showed that high concentration of crude oil inhibits germination of *Dennettia tripetala*. Similar reports were also made by Udo and Oputa (1984), Gills *et al.* (1992), Anoliefo and Vwioko (1994) and Siddiqui and Adams (2002).

Although many reports have been made on the negative effects of crude oil on plants, little or no information exists on the intraspecific differences among plants with respect to response to crude oil pollution. It is however known that different plant species responds in different ways to crude oil

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pollution. For instance while alfalfa (*Medicago sativa* L.) had significantly reduced biomass at 2% (w/w) crude oil pollution (Wiltse *et al*., 1998), Faba bean (*Vicia faba* L.) can tolerate as much as 10% (w/w) crude oil pollution (Radwan *et al*., 1998). Also, Merckl *et al*. (2004a) reported that two species of *Mimosa*, *M. orthocarpa* and *M. camporum* responded differently to crude oil pollution. The researchers also showed that different plants react differently to crude oil pollution. However most species have many accessions which have morphological and genetic differences. These differences make them to adapt and react differently to different conditions.

Soybeans are one of the most important crops in the world. They provide proteins for human consumption directly and indirectly through processed foods or livestock products. According to Oyekan (1985) the plant is one of the most important grain legumes in the world today because it plays a key role in meeting the expanding needs for protein, edible oil and calories. It can be used in a wide range of industrial, food, pharmaceuticals and agricultural products (Smith and Huyser, 1987).

Tomato enjoys worldwide distribution and is integral to the culinary disposition on multiple culture (Cox, 2000). There are different uses of tomato. It is freely eaten throughout the world. The acidic property of tomato is used to bring out other flavours; the acidic property also helps for easy preservation of tomato in homes. Tomatoes are an excellent source of lycopene, an anti-oxidant which snuffs out cancer-causing free radicals within mammalian systems. Tomato is also good source of vitamins A and C, beta-carotene, magnesium, niacin, iron, phosphorus, potassium, riboflavin, sodium and thiamine (Cox, 2000).

In Nigeria most crude oil spillages occur on farmlands reducing agricultural yields. It has also been difficult to utilize such lands as no knowledge on the performance and survival of crops on polluted soils exist. This study therefore was carried to find out the survival of different accessions of these two useful food crops (soybean and tomato) when they are grown in crude oil polluted soil. It is believed that the information obtained would help to know the appropriate accessions of the crops to be planted in crude oil polluted soil instead of allowing such polluted lands to lie fallow.

MATERIALS AND METHODS

This study was conducted in a screen house in the botanical garden of the University of Lagos, Akoka Lagos, Nigeria. The seeds of the *Glycine max* (soybean) and *Lycopersicon esculentum* (tomato) used in this study were obtained from the Gene Bank Sections of International Institute for Tropical Agriculture (IITA) and Nigerian Horticultural Institute (NIHORT) Ibadan, Nigeria respectively. The accessions of *G. max* (soybean) are TGX1019-1E, TGX 1805-1E, TGX 1440-1E and TGX 1448-2E while those of *L. esculentum* are DT95/34, DT95/370, DA99/20 and DT95/387. The accessions were selected randomly from collections of seeds in the gene bank sections of IITA and NIHORT. The choice of the two crops was based on the nutritional values has made them to be widely cultivated in the different parts of the world. The crude oil used was wellhead medium obtained from the Health Safety and Environment Laboratory of Shell Development Company, Port Harcourt, Nigeria. The soil used was sandy loam obtained from Botanical garden of the University of Lagos, Akoka Lagos, Nigeria.

The soil was sieved through a 5 mm sieve and 4000 g of the sieved soil was filled into a 5 L paint bucket. The crude oil was added into the filled paint buckets with each bucket receiving a given quantity of crude oil. The amounts of crude oil added were 25, 50, 75 and 100 g and each quantity of crude oil served as a treatment. The added crude oil was properly mixed with the soil in each bucket using hand trowel and each treatment was replicated eight times. Also eight buckets received no crude oil and served the control treatment. The treatments and the control were left for 10 days to allow for proper settling of the crude oil in the soil.

Ten seeds of each accession of the test crops were sown up to 2 cm depth into the crude oil contaminated soils in the buckets. Each bucket for each treatment received one accession of the crops and one bucket for the control also received one accession of the test crops. In all four buckets for each treatment were planted with the four accessions of *G. max* while the other four were planted with the four accessions of *L. esculentum*. The buckets were watered regularly to keep the soil moist.

The number of seedlings that emerged from each bucket was taken to be the number of seeds that germinated in such bucket. The number of seeds that germinated from each pot were summed up after eighteen days for *G. max* and *L. esculentum*. The percentage germination of each accession in each treatment was calculated using the formula:

$$\text{Germination (\%)} = \frac{\text{No. of seedlings that emerged from soil}}{\text{Total No. of seeds sown}} \times 100$$

The rate of survival of the seedlings of the plants in crude oil polluted soil was calculated for each accession in the different treatments by counting the number of seedlings that were standing after 45 days. The percentage survival of the crops was calculated using the formula:

$$\text{Survival (\%)} = \frac{\text{No. of crops that are standing}}{\text{No. of seeds that germinated}} \times 100$$

The growth rate of the plants in the crude oil polluted soil was determined by measuring the shoot lengths of the crops in the different treatments using a calibrated ruler. This was done 75 days after germination of the crops.

RESULTS

For the accessions of *G. max*, TGX 1440-1E produced the highest percentage germination in all the treatments. The highest percentage germination for the TGX 1019-1E accessions was observed in 25 g treatment while the control produced the highest germination for the other accessions of *G. max* and all accessions of *L. esculentum*. The percentage germination of the *L. esculentum* decreased as the concentration of the crude oil increased. In every treatment, the DT95/387 accession had the highest percentage germination when compared with other accessions of *L. esculentum* (Table 1, 2).

In the soil treated with 100 g of crude oil TGX 1805-31F of *G. max* had the highest survival rate with 50% survival while the TGX 1440-1E accession produced the lowest survival with no crop surviving. However, TGX 1440-1E survived more in soils with the other concentrations of crude oil than the other accessions of *G. max* (Table 3). In the case of *L. esculentum*, the DT 95/370 accession

Table 1: Percentage germination of the accessions of *G. max* (soybean) in crude oil polluted soil

Accession	0 g	25 g	50 g	75 g	100 g
TGX 1019-1E	10	30	0	20	0
TGX 1805-31F	20	0	20	0	20
TGX 1440-1E	80	70	30	20	20
TGX 1448-2E	20	40	0	20	20

Table 2: Percentage germination of the accessions of *L. esculentum* (tomato) in crude oil polluted soil

Accession	0 g	25 g	50 g	75 g	100 g
TGX 1019-1E	70	30	0	20	0
TGX 1805-31F	20	0	20	0	20
TGX 1440-1E	80	70	30	20	20
TGX 1448-2E	20	40	0	20	20

Table 3: Percentage survival of the accessions of *G. max* (soybean) in crude oil polluted soil

Accession	0 g	25 g	50 g	75 g	100 g
TGX 1019-1E	0	35	0	50	0
TGX 1805-31F	0	0	50	0	50
TGX 1440-1E	88	100	70	50	0
TGX 1448-2E	0	50	0	50	20

Table 4: Percentage survival of the accessions of *L. esculentum* (tomato) in crude oil polluted soil

Accession	0 g	25 g	50 g	75 g	100 g
DT 95/3	85	35	100	0	0
DT 95/370	85	70	100	100	50
DA 99/20	100	50	0	0	0
DT 95/387	90	100	50	0	10

Table 5: The shoot lengths (cm) of the accessions of *G. max* (soybean) grown in crude oil polluted soil

Accession	0 g	25 g	50 g	75 g	100 g
TGX 1019-1E	0	72	0	50	0
TGX 1805-31F	0	0	50	0	48
TGX 1440-1E	88	98	45	50	0
TGX 1448-2E	0	20	0	23	32

Table 6: The shoot lengths (cm) of the accessions of *L. esculentum* (tomato) grown in crude oil polluted soil

Accession	0 g	25 g	50 g	75 g	100 g
DT 95/3	46.5	4.20	1.25	0.0	0.00
DT 95/370	30.0	2.75	1.65	1.3	1.10
DA 99/20	43.0	0.50	0.00	0.0	0.00
DT 95/387	37.5	2.50	1.75	0.0	0.07

generally survived more in the crude oil polluted soils than other accessions while DA 99/20 accession showed the worst survival as it only survived in the control and 25 g treatments (Table 4).

The TGX 14440-1E accession of *G. max* produced best growth while the worst growth was produced by TGX 1448-2E. However, the TGX 1448-2E accession produced a growth trend that was highest in 100 g crude oil treatment while all the other accessions produced the worst growth in same treatment (Table 5). The best growth for all the accessions of *L. esculentum* was observed in the control treatment. However, no pattern of growth was noticed for the accessions in the crude oil treated soils as the shoots lengths of the accessions varied for each concentration. The shoot lengths of *L. esculentum* generally decreased with increase in the concentration of crude oil (Table 6).

DISCUSSION

The difference in the rate of germination is an indication that crude oil does not exhibit equal rate of toxicity to the seeds of different plants. This is similar to the findings of Merckl *et al*. (2004b) who observed that seeds of *Brachiaria brizantha*, *Centreosoma brasilinum*, *Calapagonium mucunoides*, *Desmodium glabrum*, *Panicum maximum*, *Mimosa orthocarpa*, *M. camporum* and *Stylosanthes capitata* germinated at different rates in soil contaminated with crude oil. Although the *L. esculentum* exhibited higher germination in the control experiment than the *G. max*, the higher percentage germination of *G. max* observed in the treatments suggests that seeds of *G. max* survive more in soils polluted with high concentration of crude oil than *L. esculentum*. The generally low rate of germination of the crops observed in this study could be due to the general unfavourable condition that crude oil creates in soils (Merckl *et al*., 2004b).

The non germination of the crops in soils with 100 g crude oil treatment indicates acute effect of crude oil on seeds and that such concentrations are toxic to the seeds of the crops (Agbogidi *et al*., 2006). This is similar to the failure of germination of seeds exposed to high concentration of crude oil reported by Agbogidi *et al*. (2006) and Siddiqui and Adams (2002). According to Baker (1970) and

Chaineau *et al*. (1997), oil coat on seeds can prevent oxygen and water uptake which are essential for germination. Merckl *et al*. (2004b) also suggested that the toxic nature of crude oil and the unfavourable soil moisture condition created by oil pollution could inhibit germination of seeds. The non germination of the seeds could be attributed to oxygen tension in soil contaminated with crude oil which could have affected the respiratory system of embryo and hence, the viability and germination of the seeds (Coleman and Crossley, 1996). Oil components once inside seeds can alter metabolic reactions or kill the embryo of such seeds thus preventing such seeds from germinating (Baker, 1970; Udo and Fayemi, 1975). The failure of seeds to germinate in soils with high concentration of crude oil could be the cause of slow rate of revegetation caused by crude oil that was reported by Kinako (1981).

The inverse relationship between the rate of germination and the concentration of crude of crude oil noticed with TGX 1440-1E accession of *G. max* and all the accessions of tomato in this study is in conformity with the findings of Merckl *et al.* (2004b) who reported that *C. mucunoides* and *D. gabrum* germinated in inverse relationship with the concentration of crude oil applied to soil. This trend of germination had also been reported by Salanitro *et al*. (1997), Bossert and Bartha (1985) and Gallego Martinez *et al*. (2000). Similar observations were made by Siddiqui and Adams (2002) who reported that germination of ryegrass was inhibited by the presence of low molecular mass hydrocarbon in diesel. The reduced seedling emergence in crude oil polluted soil could be due to toxic effects of oil or by unfavourable soil moisture conditions (Merckl *et al*., 2004b).

The higher rate of germination in high concentration of crude oil produced by some accessions of *G. max* (TGX1019-1E: 75 g>50 g; TGX1805-31F, 50 g>25 g and 100 g>75 g and TGX1448-2E, 75 g>50 g) is similar to the promotion of the rate of germination of *M. orthocarpa* in crude oil polluted soil observed by Merckl *et al*. (2004b). It also conforms with the results of Prado *et al*. (1994) who worked on cowpea in crude oil polluted soil.

Although there was high rate of survival of *L. esculentum* in the polluted soils this may not make one to conclude that it grows well in crude oil polluted soil. In addition, when the rate of germination is considered together with the survival levels it shows that most times only one crop stand was noticed in the buckets containing *L. esculentum*. The higher survival of DT 95/370 in all the concentrations of crude oil than the other accessions is an indication that all the accession do not have equal rate of survival and that DT 95/370 tolerates crude oil pollution more than other accessions of *L. esculentum*. The difference in the rate of survival we observed in this study is also an indication that different crops have different resistance to crude oil pollution even if such plants belong to same species.

The death of plants after germination noticed in this study conforms to the finding of Merckl *et al*. (2005a) who reported death of *C. mucunoides*, *C. brasilianum* and *S. capitata* six weeks after germination. According to Merckl *et al*. (2005b) the reason for the death of the plants after germination noticed in this study could be continual exposure of the crops to crude oil in the soil. The longer exposure of plants to contaminants prolongs the toxic effects of such contaminants to plants hence death of crops in such situation. Also, Merckl *et al.* (2005b) were of the view that oil causes rapid drainage of soil water leading to limited moistening effect in the root area. This could also lead to death of the crops after germination as was noticed in this study. The germinated seedlings could have died as a result of the uptake of phytotoxic compounds (Wiltse *et al*., 1998) present in the crude oil. Although TGX 1440-1E did not survive in the 100 g crude oil treated soil, its greater survival in the other treatments suggests that it tolerates crude oil pollution than the other accessions of *G. max*. The slight difference in the rate of survival of other accessions of *G. max* in crude oil may be an indication that different accessions of *G. max* react to crude oil pollution in different ways. The greater number of death of the *G. max* than the *L. esculentum* reported in this study could be as a result of the small size of the containers used in the study (Merckl *et al*., 2005a). Since the *G. max* geminated more and grew faster than the *L. esculentum*, it required more space for the roots to grow deeper and escape from the constant exposure to crude oil pollution (Merckl *et al*., 2005b). Therefore the small size of the containers used in this study could have restricted the escape of the roots from the polluted zone. As shown by the results, the accessions of *G. max* grew faster in the crude oil polluted soil than the accessions of *L. esculentum*. This may be an indication that *G. max* may have better adaptation to crude oil pollution than *L. esculentum*. The possible explanation for this is that as legumes, *G. max* can fix nitrogen to the soil thereby reducing the effect crude oil pollution will have on its growth. This reduces competition with microbes and other plants for limited supplies of available nitrogen as oil-contaminated sites (Merckl *et al*., 2004a). Also, according to Kirk *et al*. (2002), the legumes have roots which can penetrate deep into the soil thereby moving away from the constant effect of crude oil in the polluted soil. The generally poor growth rate of *L. esculentum* in the polluted soils suggests that the crop does not grow well in crude oil polluted soil.

The growth of the accessions of *G. max* in the different treatments indicated that best growth of *G. max* occurred in the soils treated with 25 g crude oil. For the accessions of *L. esculentum*, the best growth occurred in the control treatment. The trend of growth of *G. max* is similar to the findings of Akinola *et al*. (2004) while the trend of growth of *L. esculentum* is an indication that even low concentration of crude oil adversely affects the growth of the crop. The difference in the growth rate of the accessions of *G. max* and *L. esculentum* is an indication that even within a plant species the level of effect of crude oil produced differs from one accession to another.

The combination of results of the percentage germination, percentage survival and growth obtained in this study showed that TGX 1440-1E accession of *G. max* has best potential of growing in crude oil polluted soil than the other accessions of *G. max* and *L. esculentum*. This is considering the fact that its seeds germinated in all the treatments, it survived in three out of the four treatments and it produced the longest shoot length in all the treatments. Its non-survival in 100 g crude oil treated soil may imply that the treatment is toxic for the plant to grow in. According to Siddiqui and Adams (2002) the phyotoxicity of oil increases with the quantity of oil spilled. Also, the 100 g treatment could have led to poor aeration of the soil thereby reducing the activities of the microbes in the rhizosphere. According to Kapulnik (1996) the rhizosphere microbes can promote plant health by stimulating root growth and by enhancing water and mineral uptakes. Therefore the prevention of proper functioning of the microbes as result of the inhibition of aeration by high concentration of crude oil in the soil would have affected the growth and survival of *G. max* in the crude oil contaminated soil. This can be used to explain why most of the accessions did not survive in the 75 and 100 g treatments. It should be noted that although DT95/370 accession of *L. esculentum* survived in all the treatments, its very poor growth rate in crude oil treated soils may mean that it does not have the ability to grow in crude oil polluted soil.

CONCLUSION

The study of the accessions of *G. max* and *L. esculentum* for germination, survival and growth revealed that the different accessions of these crops have different levels of tolerance to crude oil polluted soil. The different levels of tolerance are an indication that although plants may belong to same species they may not be of same quality. Also, the ability of all the accessions to germinate and grow in the sandy-loam soil used for this study indicates that such crops can grow in a typical Niger Delta region of Nigeria where most oil exploration activities take place.

This study also showed that the accessions of *G. max* grow better than the accessions of *L. esculentum* in crude oil polluted soil. Further studies can also be conducted to ascertain that the seeds of *G. max* do not accumulate crude oil and or its degradation products. This will ensure that the consumption of plants does not lead to poisoning of man when such seeds from crude oil polluted soils are consumed. The study also revealed that the different accessions of each of the crops the responded differently to crude oil pollution and that the TGX 1440-1E accession grows better in crude oil polluted soil than the other accessions investigated in this study. The differences by the different accessions of crops show that there are intraspecific differences on the response of plants of same species to crude oil pollution. The potentials exhibited by TGX 1440-1E to grow well and survive in crude oil polluted soil may be used to screen the accession on its ability to clean up crude oil polluted soil. It is our suggestion therefore to try the accession for phytoremediation studies in crude oil polluted soil and also to plant it in crude oil polluted soils instead of leaving such soils fallow. This will help to eliminate wastage of such polluted soil, provide cover to the polluted so that contact and ingestion of the polluted soil by children will be minimized and to get other values of the crop apart from its widely publicized nutritional values. If the plant is found to be a good phytoremediator of crude oil polluted soil, it can be used to cleanup soil polluted with crude oil. This will add to the already known uses of the plant. Hybridization study between the 1440-1E and the TGX 1805-31F accessions of *G. max* can also be carried out to determined whether a hybrid from the two accessions can survive and grow more than the others in crude oil polluted soil.

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