GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(*Published By*: Global Institute for Research & Education)
www.gifre.org

DOSE-RESPONSE STUDY OF PLANTS (*GYCINE MAX L., VIGNA SUBTERANEA L.* AND *ZEA MAYS L.)* TO DIESEL OIL POLLUTION

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ABSTRACT

Field trial was carried out in 2010 Cropping Season to determine the growth and development of Bambaragroundnut, maize and soybean as influenced by diesel oil pollution in loamy sand soil at Teaching and Research Farm of Faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri. The experiment was a split-plot design based on Randomized Complete Block design and least significant difference was used in separating the means. The crop plants (Bambaragroundnut, maize and soybean) constituted the main plots and the diesel oil pollution levels (0, 1.0, 1.5 and 2.0 litres) constituted subplots and each treatment was replicated five times. Results showed that diesel oil pollution at different levels (0, 1.0, 1.5 and 2.0 litres) significantly affected the performance of Bambaragroundnut, maize and soybean. The result obtained from the current investigation indicates that the test crops exhibited dose-dependent response to the diesel oil polluted soils. Reduction in percentage germination, plant height, leaf area, leaf area index, leaf area ratio, due to effect of high dose (2.0 litres) diesel oil pollution were significantly different ($P<0.05$) compared to their control. However, Bambaragroundnut performed significantly better in terms of percentage germination in control treatments than maize and soybean, whereas higher diesel oil pollution affected germination severely in bambaragroundnut than in maize and soybean. It was observed that 2.0 litres reduced the plant height significantly compared to the control and other treatment levels. However, soybean performed well in plant height and leaf area in all treatments levels at early growth stages compared to maize and bambaragroundnut whereas at early maturity stage, maize plant performed significantly well. The study also indicated that soybean in all treatments levels recorded significantly higher leaf area index than bambaragroundnut and maize. However, maize recorded significantly higher yield at all the treatment levels than observed in soybean and bambaragroundnut. Hence, diesel oil pollution has potential for reducing growth and development of crops

Key words: Dose-Response,dieseil oil, Pollution,*Glycine max,Vigna subterranean,Zea mays.*

INTRODUCTION

The emergence of crude oil industries has contributed immensely to changing the state of Nigeria Economy and the environment. The oil industry is a major source of environmental pollution and its adverse ecological impacts have been reported (Ibia *et al.,* 2002; Ekpo and Thomas, 2007). This is wide spread with specifically more serious damage on the oil producing areas of Niger Delta. Crude oil and refined petroleum product pipelines pass through these communities to their target destination, with the current high rate of pipeline vandalization in Nigeria, these communities and their environment suffer various degrees of damages arising from oil spillages. Furthermore, accident during transportation, pipeline leakages and ruptures has been made known in various media reports (Ehiagbonare *et al.,* 2011).

In Niger Delta area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment (Nwaogu *et al.,* 2008). Oil spills in coastal areas cause immediate and obvious problems to animals and plants. There are also long term effects on ecosystems related to the release of toxic components over a prolonged period as the oil breaks up and concentration of toxicants in organisms towards the top of the food chain increases (Samanta *et al.,* 2002).

Petroleum contamination also results from leakage above ground and underground and storage tanks, spillage during transport of petroleum products, abandoned manufactured gasoline sites, other unplanned releases and current industrial processes (Mishra *et al.,* 2011, Sarkar *et al.,* 2005).

Petroleum compounds are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature and low volatility and thus they pose a significant threat to the environment (Abed *et al.,* 2002). The constituents of these contaminants such as diesel oil, are carcinogenic, mutagenic and are potent immune-toxicants, thus posing a serious threat to human and animal health (Boonchan *et al.,* 2000; Samanta *et al.,* 2002).

Diesel oil can enter into the environment through leakage from storage containers, refueling of vehicles, wrecks of oil tankers and warships carrying diesel oil and through improper disposal by mechanics when cleaning diesel tankers (Njoku *et al.,* 2009).

Adekunle *et al.* (2003) stated that the Niger Delta Area of Nigeria is the hardest hit of environmental destruction arising from oil production. It further stated that people living in the Niger Delta, Area are faced with health hazards, lack of safe drinking water to uncultivable land.

Frequent crude oil spillage on agricultural soils, and the consequent fouling effect on all forms of life, renders the soil (especially the biologically active surface layer) toxic and unproductive. The oil reduces soil fertility such that most of the essential nutrients are no longer available for plant and crop utilization (Abii and Nwosu, 2009).

Maize (*Zea mays,)* and Soybean (*Glycine max)* are important food, fodder and industrial crops (Food and Agricultural Organization, 2007). They are widely cultivated, consumed and service a number of industries as raw materials. Bambara groundnut (*Vigna subterranean*) has long been food crop and is widely cultivated in Africa. It is also known to compete with soybean in area of protein needs of farmers in sub-Sahara Africa and recently it has become of interest to researchers on it productivity.

MATERIALS AND METHODS

Field experiments to study Assessment of root growth characteristics of Soybean *(Glycine max L.)* were conducted during 2010 planting season at the Teaching and Research Farm of Imo State University..

The Experimental Site: The experimental site was located at the Teaching and Research Farm of the Faculty of Agriculture and Veterinary Medicine, Imo State University, Owerri. Owerri lies between the latitudes $5^0 10^1$N and $6^0\ 0^1$N and longitudes $6^0 35^1$E and $7^0 0^1$E within the southeast rainforest agricultural zone of Nigeria.

Source of Plant Materials and Diesel: Certified seeds of soybean were purchased from Imo State Agricultural Development Programme (IMOADP). The diesel oil was purchased from Texaco Fueling Station Okigwe Road, Owerri, Imo State

Treatment application: The land was manually cleared. The soil was tilled and raised to beds of 1m x 1m x 0.75m Different levels (0.0, 1.0, 1.5 and 2.0litres) of diesel oil contaminant were poured on the beds which was then incorporated by pulverizing the soil using a hand trowel to achieved even distribution of the diesel oil. It was left for 7days before planting in order to enable the volatile substances which are toxic to plant, escape into the atmosphere.

Planting: The healthy seeds of soybean were used. The soybean seeds were sown at a depth of 2cm with spacing of 10cm x 10cm at the rate of 2 seeds per hole with total of 10 stands per bed which was thinned down to five seedlings per bed 14 days after germination to attain seedlings of equal vigour.

Sampling Procedure: Plant samples were destructively sampled at 2 weeks interval during the growth period for the following parameters percentage emergence were calculated using the formular below:

$$\frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seed Planted}} \quad \frac{\times 100}{1}$$

Plant Height (cm): Plant height was measured from the soil base level of the stem to the top or terminal bud using a meter rule.

Leaf Area (cm^2) of Soybean: Leaf area of soybean plant was calculated by measuring the length (L) and width (W) of the soybean using a general formular, $LA = 6.532 + 2.045\ (L_1W_1)$ for leaf area calculation as proposed by Ogoke *et al.* (2003).

Leaf Area of Maize (Cm^2): The leaf area of maize was estimated using the formular leaf area = LXB (0.75) as described by Ogoke *et al.* (2003).

Leaf Area(cm^2) of Bambaragroundnut: The leaf area was calculated using the formular LA = Total terminal leaflet area x 1.8 (Sessay and Sungu, 2000).

Leaf Area Index: It was calculated for all the test crops using the formular according to Akonye and Nwauzoma, (2003).

$$\text{LAI} = \frac{\text{Leaf area of plant (cm}^2\text{)}}{\text{Land area covered by individual plant (cm}^2\text{)}}$$

Leaf Area ratio (cm^2g^{-1}): This was calculated for all the test crops using the formular below as described by Kang & Van (2004).

$$\frac{\text{Total Leaf Area (cm}^2\text{)}}{\text{Total dry weight of the plant (g)}}$$

STATISTICAL ANALYSIS

The data collected from the study were statistically processed on computer using Excel XP, SPSS 13.0 difference among treatments were tested using analysis of variance followed by Duncan multiple range test.

RESULTS

Percentage Germination (%)

Statistical analysis showed significant difference ($P<0.05$) between the levels of pollution with diesel oil on germination of the test crops. 2.0 litres of pollution level significantly reduced germination of Bambaragroundnut (10%), maize (20%) and soybean (25%) when compared with the control (Bambaragroundnut 80%, maize 78% and soybean 50%). The germination of bambaragroundnut was more adversely affected by diesel oil pollution than soybean and maize (Fig. 1).

Plant Height(cm)

The result of plant height as shown in figure 2 revealed that the control (0.0litre) had the highest mean plant height at all the growth stages. While the lowest was observed in 2.0 litres the difference in height was found to be very significant ($P<0.05$). However, at 2, 4 and 6WAP, in all the treatment levels, soybean performed significantly better than Bambaragroundnut and maize whereas at 8 and 10WAP maize plant recorded significantly highest plant heights at all the treatment levels which was significantly compared to Bambaragroundnut and soybean

Leaf Area (cm^2)

In figure 3, 2.0litres recorded lowest leaf area in Bambaragroundnut, maize and soybean, at 2, 4, 6, 8 and 10WAP compared to the highest leaf area observed in control (T_1). However, among the crop species, soybean recorded highest leaf area in control plots up to 8WAP compared to maize and Bambaragroundnut. At the end of the growth period (10WAP), maize plant was found to have higher LA with 1345.9cm^2, 138.3cm^2 and 1581.3cm^2 recorded for 1.0l, 1.5 and 2.0litres respectively while the lowest LA of 216.3cm^2, 41.7cm^2 and 0.000cm^2 at 1.0, 1.5 and 2.0 litres were recorded for bambaragroundnut (fig.3).

Leaf Area Index

In the figure 4, soybean significantly recorded the highest leaf area index at all treatment levels compared to the lowest leaf area index recorded in Bambaragroundnut and maize at 2, 4, 6, 8 and 10WAP. Control treatment recorded the highest leaf area index compared to T_4 diesel oil treatment levels at all growth stages.

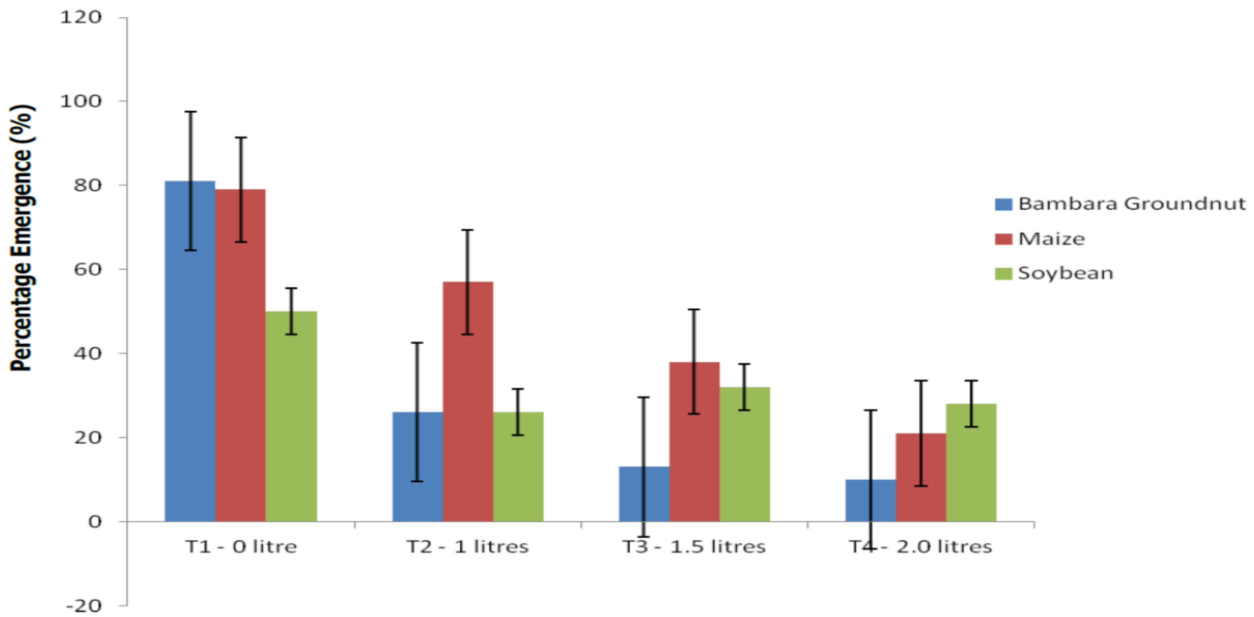
Leaf Area Ratio (Cm^2g^{-1})

The result in figure 5, showed that, at 2,4,6,8 and 10WAP, control gave significantly higher leaf area ratio compared to bambaragroundnut, maize and soybean. However at 8 WAP, soybeans performed significantly better than bambaragroundnut and maize in control 1.0 and 1.5 litres. Whereas in 2.0litres, at 8 and 10WAP, maize plant significantly recorded higher leaf area ratio than leaf area ratio recorded in soybean and bambaragroundnut respectively.

Yield of Test Crops as influenced by diesel oil

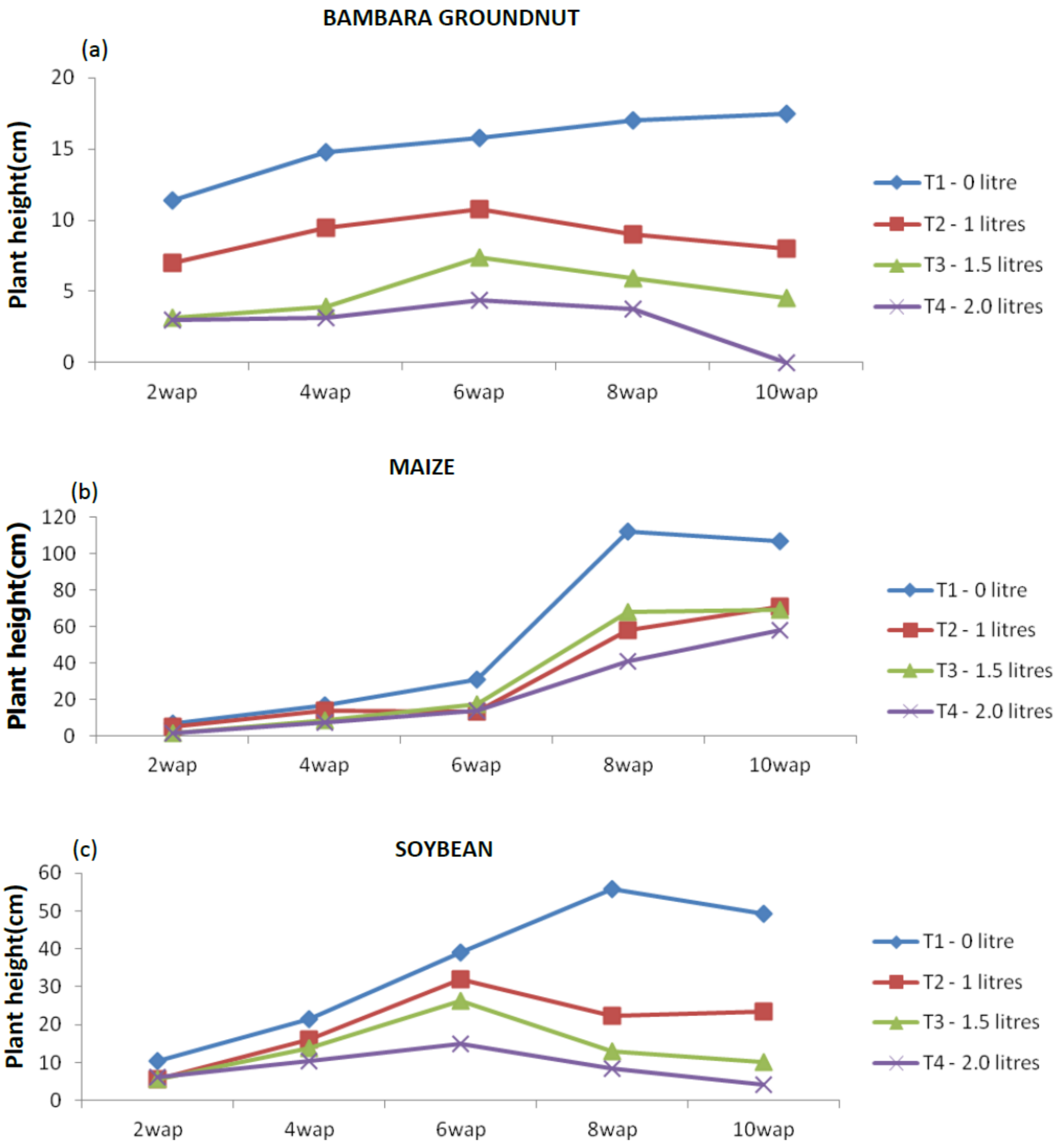
In figure 6, 2.0 litre pollution level caused a marked reduction in yield of the test crops which was significantly difference ($P<0.05$) compared to the control treatment. However, maize plant significantly produced higher yield in control 27.28kg/ha than soybean (1.818kg/ha) and Bambaragroundnut (5.744kg/ha).

However, the same trend was observed in 1.0, 1.5 and 2.0litres pollution levels. It was observed that diesel oil pollution impacted heavily on the yield of Bambaragroundnut than soybean and maize.



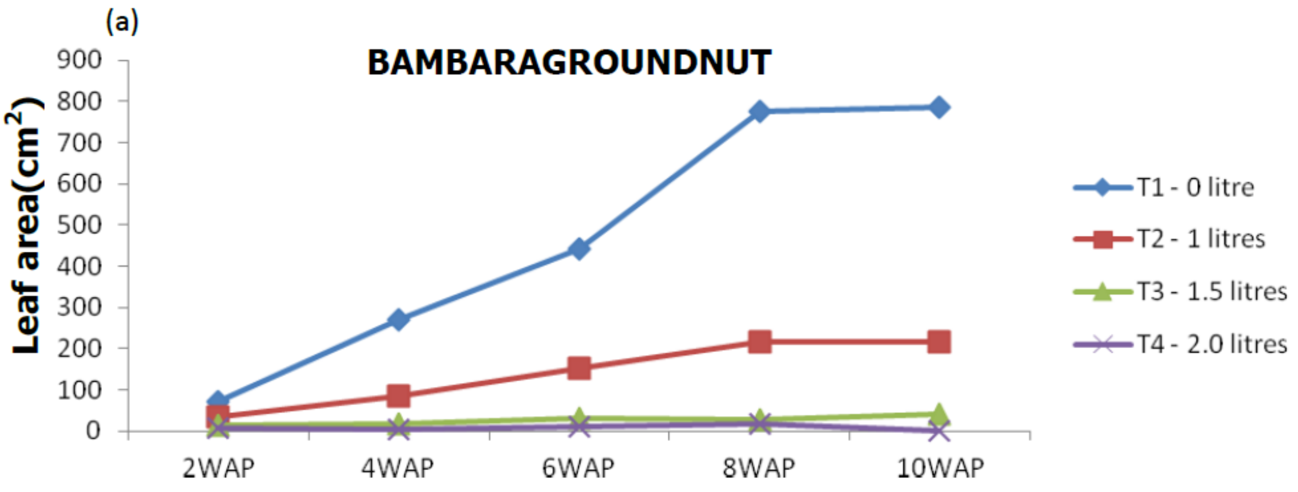
Treatment levels

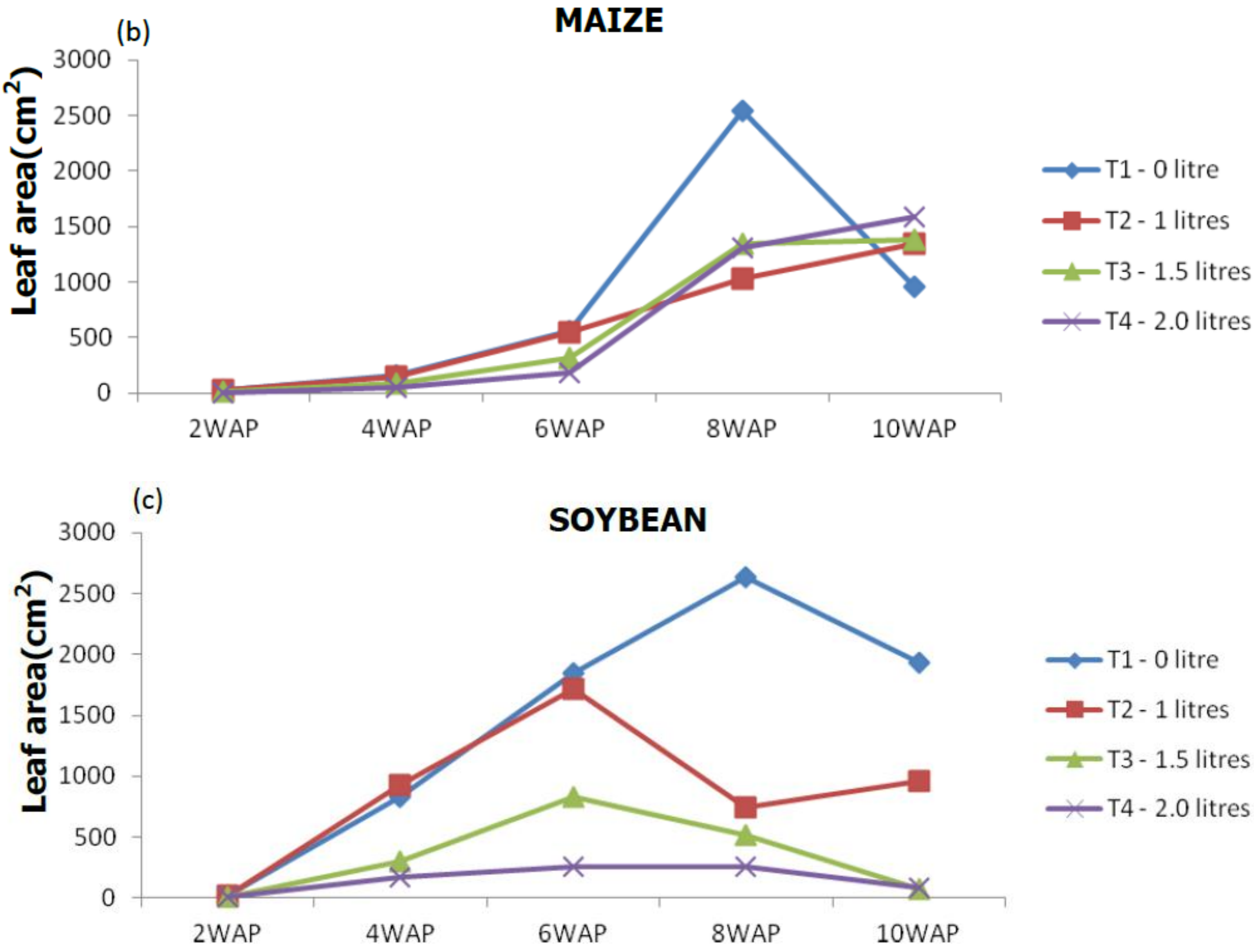
Fig.1: Percentage Emergence (%) of Test crops as influenced by diesel oil pollution



Weeks After Planting(WAP)

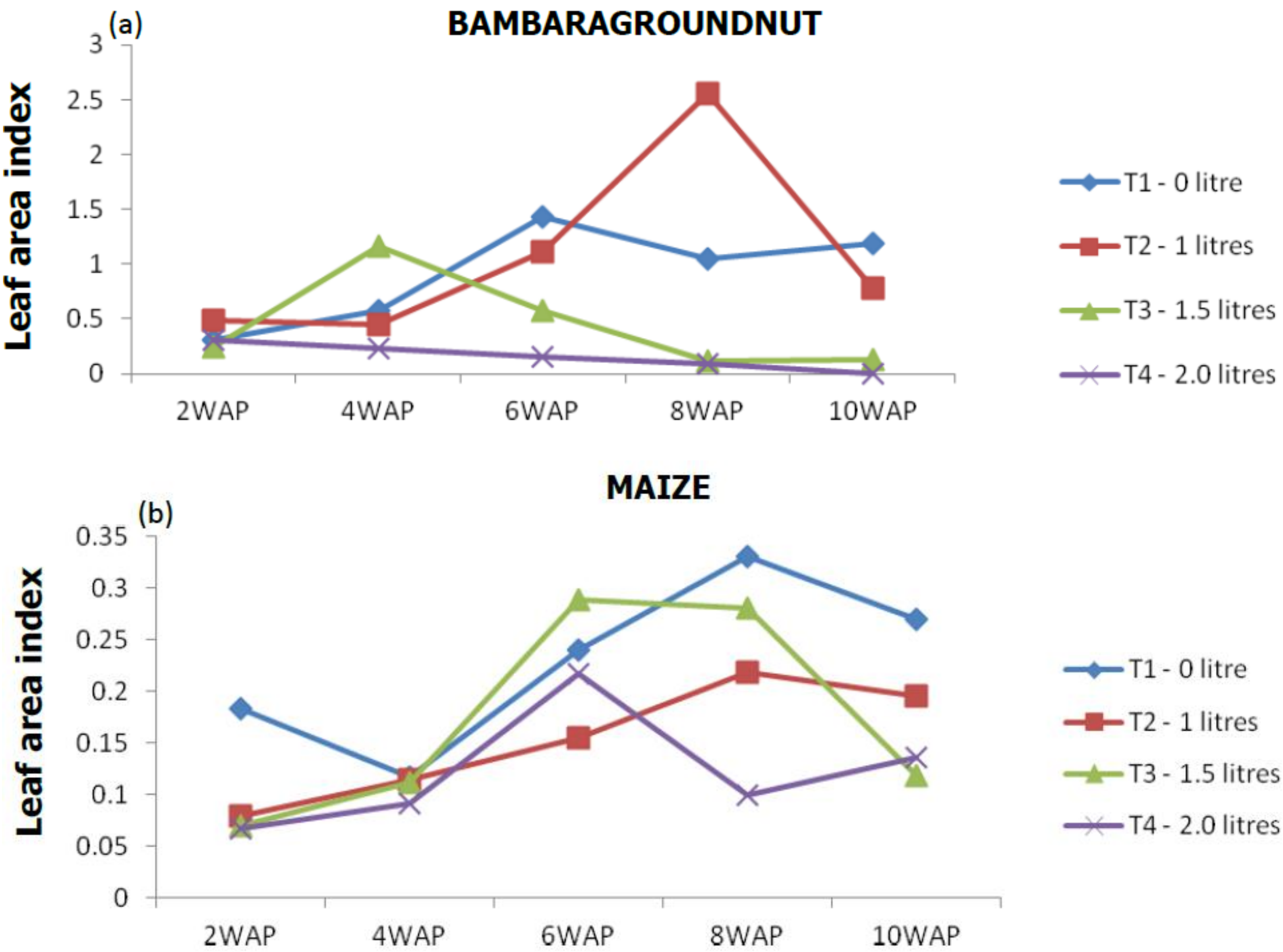
Fig. 2 (a, b, and c): Plant heights(cm) of Test crops as influenced by diesel oil pollution

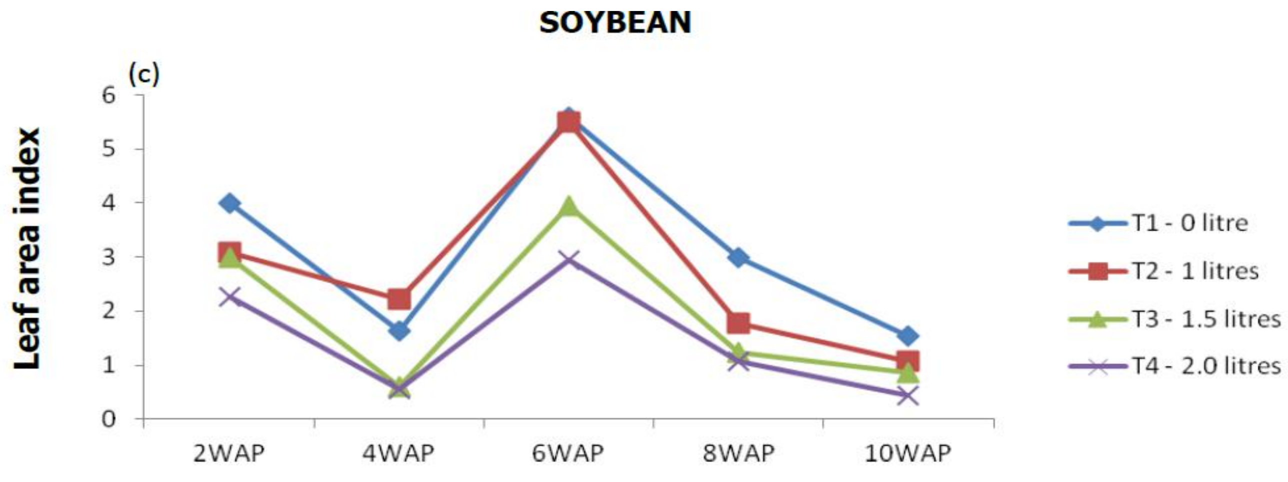




Weeks After Planting (WAP)

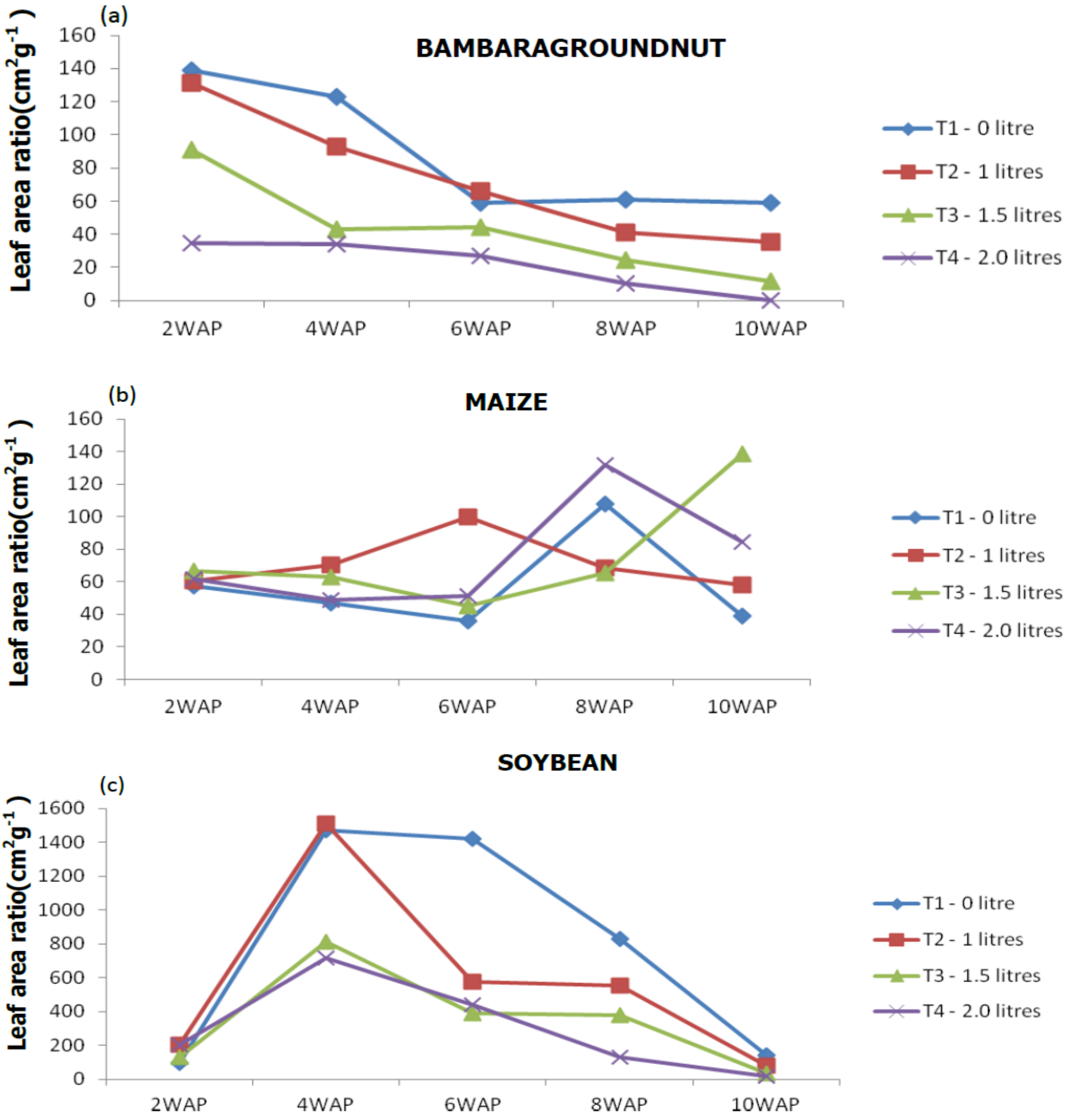
Fig. 3 (a, b, and c): Leaf area (cm^2) of Test crops as influenced by diesel oil pollution





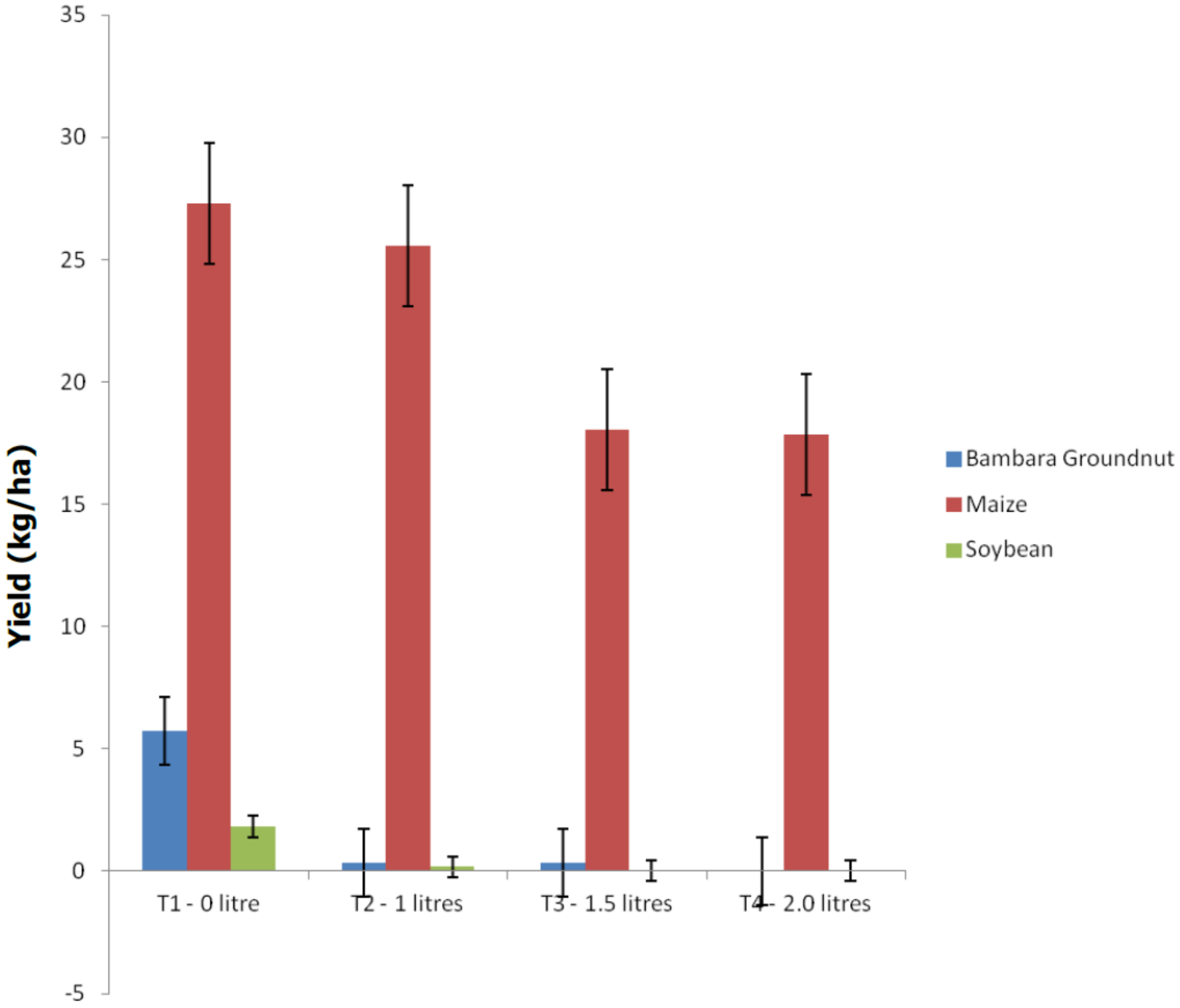
Weeks After Planting (WAP)

Fig.4 (a, b and c): Leaf area index of Test crops as influenced by diesel oil pollution



Weeks After Planting(WAP)

Fig. 5: (a, b and c): Leaf area ratio(cm^2g^{-1}) of Test crops as influenced by diesel oil pollution



Treatment level

Fig. 6: Yield (kg/ha) of Test Crops as influenced by Diesel oil pollution

DISCUSSION

Effects of different levels of diesel oil pollution on crop species significantly influenced the percentage emergence. It was observed that growth of test crops under control (T_1) recorded higher percentage emergence compared to the lower percentage emergence observed in T_4 (2.0 litres) interactions with test crops. Furthermore, it was observed that Bambaragroundnut in control recorded the highest percentage germination of 80%, whereas in the diesel oil polluted soils, maize plant in T_2 recorded the highest (51%) percentage germination. The effect of T_4 (2.0 litres) pollution level on test crops, showed that Bambaragroundnut was drastically affected compared to the maize and soybean. This observed difference by the crops could be due to variability in crop specie and different concentration of diesel oil in the soil. This supported the finding of Pyteleroski *et al.* (1981), and Wyszkowska and Kucharski (2000). A similar effect was also observed by Adam and Duncan (2002), who reported reduction in germination rate in several plant species mainly in commercial crop caused by petroleum contamination. Also the level of contamination determines the extent of damage and also inhibition. At high levels of contamination (1.5litres and 2.0 litres) although there was germination in all the crop plants, there was a reduction in the seed germination of these test crop plants and this affected their heights. This indicates that the effect of lethal concentrations of petroleum contaminants to different plants vary. The presence of oil and level of contamination in the soil affects germination and subsequently growth of plants in such soil (Terge, 1984). The effect could also be as a result of formation of polar compounds dissolved in water that could penetrate the seed coat, exerting polar narcosis (Wang *et al.*, 2001; Adam and Duncan, 2002).

This trend was also recorded for six agronomic plants by Issoufi *et al.* (2006). They recorded reduced seed germination as the level of contamination increased for plants like corn, perennial nyegrass, wheat, alfaffa, hairy vetch and soybean. The results in this study also agreed with the works of Anoliefo and Vwioko (1995), and Anoliefo and Edegbai (2000) that hydrocarbons can inhibit the growth of plants.

The differences in plant height among the crop species response arose largely because of the difference in the genetic makeup of plants since they are not from the same family. However, the maize was found to have slow growth at 2 to 6WAP compared to the soybean plant but showed a steady increase in performance towards the maturity stage. On the other hand, soybean plant which is in the same family with Bambaragroundnut (legumes) showed significantly greater height than Bambaragroundnut throughout the experiment. This difference could be due to inherit ability of each of the crop to tolerate diesel oil pollution levels. This findings or inference agrees with that of April and Sims (1990).

As expected, different levels of diesel oil concentration significantly affected plant heights. The control (no pollution), performed well in terms of plant height than T_4 (2.0 litres pollution level). This reduction in plant height due to higher (severe) diesel oil concentration could be due to reduction in soil nutrients such as nitrogen necessary for plant growth occasioned by diesel oil pollution. This was similar to finding in the effect of spent oil on *Amaranthus hydridius* (Ojeba and Sadiqi, 2002). Njoku *et al.* (2008) also found similar findings on the effect of crude oil on the growth of accessions of *Glycine max* and *Lycopersicon esculentum.*

It was observed in this result that control treatment recorded significantly higher plant heights than other levels of treatment. Maize plant shows high degree of tolerance going by the heights recorded towards the end of maturity. This was in contrast to bambaragroundnut and soybean which showed significant reduction towards the maturity. This could be attributed to genetic diversity among the crop used in this research. It was observed that irrespective of specie of plant, growth of test crops in diesel oil polluted soil significantly reduced plant height in all the plants. This could be due to a disruption in aeration, biological and physical properties of the soil which affect the growth of plants. It corresponds with report of Asquo *et al.* (2002) on effect of oil pollution on okra and fluted pumpkin.

Therefore, it is important to mention here, that plant height as a plant growth parameter and yield is vital for plants. This is because; the taller a plant, the higher the amount of light energy absorbed by such plant and invariably, the higher the amount of light, the higher rate of photosynthesis and consequently the amount produced by the plants.

At lower concentration, growth was stimulated; this could be attributed to higher microbial activities and nutrient availability due to reduced toxicity of diesel oil in the soil compared to the higher diesel oil concentration. At different levels of contamination, the growth and performance of a crop plant will vary greatly. At high concentration these contaminants can kill or severely impede plant growth and germination (Kirk *et al.,* 2002).

The leaf area also determines the productivity of a crop. It was observed that the leaf area was affected significantly by different levels of diesel oil contamination and also due to crop specie response.

The level of damage or reduction in leaf area as evident from this study showed that in higher levels of contamination, 1.5 to 2.0 litres were most damaging to the leaf area in all the plants used. This could be attributed to shrinking of leaf size due to physiological and anatomical aberration caused by diesel oil in contact with cells which reduced expansion of leaves. This is in agreement with the work of Agbogidi *et al.* 2005) the differential changes in the rate of leaf growth may be associated with anatomical and morphological changes caused by oil.

Leaf area performance of test crops in interaction with different levels of diesel oil showed degree of variability as shown in figure 3, revealed that the test plants achieved higher leaf area in control treatments than other treatments levels (1.0, 1.5 and 2.0 litres of pollution). In this study soybean crop plant had a greater leaf area than Bambaragroundnut while maize plant at 10WAP showed higher leaf area followed by soybean and Bambaragroundnut.

It was observed that at initial stage of the experiment, soybean performed creditably well irrespective of the condition but could not withstand nutrient depletion whereas maize plant could withstand or tolerate adverse conditions occasioned by diesel oil pollution. Therefore, degree and variability of response to diesel oil pollution is genetical. This agreed with the findings of Ehiagbonare *et al.* (2011). Also Salanitro *et al.* (1997) observed that the toxicity effect may not just be that arising from the diesel fuel contamination but other factors like soil properties, hydrocarbon type, microbial load and genetic diversity in plant communities.

Generally, leaf area was reduced drastically in test crops by higher (2.0 litre) pollution levels. The observed reduction in the leaf area at higher levels of oil application may be attributed to physiological drought cause by diesel oil which creates conditions that limited water supply to the plants. Cutler *et al.* (1987) reported that water stress limits leaf development primarily through a reduction in cell size rather than cell number in *Nicotiana* tobacum and *Gossypium arboretum.* Another possible explanation to reduce leaf area could be attributed to the fact that the diesel oil formed a hydrophobic layer over the root, which limited absorptions of water and nutrients (Omosun *et al.,* 2008).

Leaf area index gives understanding of the interaction between crop growth and environment. In this study the soybean plant showed greater response in terms of leaf area index at 2,4 and 6WAP compared to the Bambaragroundnut and maize plant. This could be attributed to degree of variability in genetic constitutes of crop plants used in this research. Also it could be attributed to absorption of nutrients necessary to development of leaf canopy. However, greater leaf area index was recorded in Bambaragroundnut at 8WAP. This fluctuation could be due to physiological state of plant in relation to time of harvest. It was found in this experiment that diesel oil contamination decreased the leaf area index of crops. 2.0 litre of diesel oil (T_4), which was the highest pollution level decreased the leaf area index irrespective of time of analysis in the test crops, compared to the control treatment (T_1). This showed that the presence of diesel oil in the soil significantly decreased the available forms of Nitrogen, phosphorus and potassium to plants. This agrees with the findings of Dimitrow and Markow (2000). However, degree of reduction in leaf area index was minimal in T_2 (1.0 litre level) in contrast to T_3 and T_4 respectively. This implies that at low level of diesel oil pollution (T_2) the test crops were able to assessed nutrients due to high microbial activities in such soil which in turn made essential nutrients readily available for canopy development. Anoliefo and Ediegbai (2009), reported that low level of soil pollution could be easily be degraded by natural rehabilitation in soils, increase organic matter in soil and improve the fertility, physical and chemical properties of the soil.

The leaf area index production per plant increased in the test crops interaction with control and decrease in interaction with increase in concentration of diesel oil pollution in the soil. However, the rate of leaf area index production varies due to interactive ability of individual test crops with levels of diesel oil pollution (Figure 4.). In this investigation soybean gave highest leaf area index in all the interaction levels compared to the Bambaragroundnut and maize. This implies that these crops have natural variations in their ecological and biological characteristics. The result of this study from physiological view indicates that soybean can tolerate diesel oil pollution than Bambaragroundnut and maize, also has potential for phytoremediation.

It was observed that at 10WAP, Bambaragroundnut recorded zero in LAI. This was as result of death of plants occasioned by phytoxocity of higher concentration of diesel oil (2.0 litres) pollution. According to Wyszkowski and

Zoikowska (2008), proper growth of cultivated plants is dependent on the content of nutrients in the soil. The inhibition of the growth of Bambaragroundnut observed in this study occurred due to the effect of diesel oil has on soil. The reduction of LAI towards maturity stage (10WAP) could be attributed to exhaustion of nutrient from the sandy loam soil used in this study by the plants from the contaminated soil and uncontaminated soils (control).

The leaf area ratio results revealed that, soybean plant performed better or produced higher leaf area ratio at all the growth stages than the Bambaragroundnut and maize as shown in figure 5. This higher species response on leaf area ratio by soybean, depicted level of tolerance, photosynthetic efficiency and adaptability to diesel oil polluted soil.

In this study it was observed that low concentration (T_2) diesel oil pollution at 2 and 4WAP have the higher leaf area ratio than recorded control. This could be attributed to high microbial activities associated with low level of diesel oil contamination which made nutrient available for leaf expansion. Similar observation of increased leaf area ratio in the presence of light doses of oil contamination has been made by Bamidele and Agbogidi (2000).

In the present investigation, significant leaf area ratio differences were observed among diesel oil levels and interaction levels with soybean, Bambaragroundnut and maize. The figure 5 indicated that application of 2.0 litre diesel oil pollution recorded significantly lower leaf area ratio in Bambaragroundnut, maize and soybean, this reduction could be due to the difficulties in absorption of water and nutrients by roots as well as toxicity of the diesel oil components. This poor growth is in conformity with findings of Asquo *et al.* (2002).

However, the deaths of seedling recorded at 10WAP in higher diesel oil pollution level by Bambaragroundnut plant and drastic reduction in the leaf area ratio in the test plants on the polluted soil showed the persistence effect of diesel oil pollution in an environment irrespective of amount of rainfall and temperature. These findings disagree with findings according to Pezeshki and Delaune (1997), who stated that effect of crude oil on plants could be short term under field conditions, since plants would likely recover once residual oil is removed by rainfall or tidal action.

The reduction in the yield recorded in this study irrespective of crop species could be attributed to the reduction recorded in the macro-elements analysed in the plant tissues. It has been said and confirms in this study that diesel oil pollution decreases the availability of nutrients in the soil (Baran *et al.,* 2002). The reduction in the yield and yield component also could be attributed to reduction in the leaf area, leaf area index and leaf area ratio recorded in this study. The extent of leaf production in plants spite of the environment determines the photosynthetic metabolism /activities which either decrease or increase the plant yield. According to Kathirvalan and Kalaiselvan (2007), the crop yield is ultimately determined largely by the leaf surface area. Therefore, the reduction in leaf area as observed in this study implies that there would be a low photosynthesis efficiency of the plant, as much of the solar energy emitted by sun would not be absorbed by the plant for photosynthesis. This could have led to low yield of the plant as was observed in this study.

However, the reduction in the yield, depend on the level of diesel oil pollution, plant species, soil conditions and other environmental factors operating at the time of experiment. Some researchers (Wyszkowski and Wyszkowska, 2005; Ogboghodo *et al.*, 2004; Kaimi *et al.,* 2006) reported that a negative effect of crude oil derivatives on plant yield depends not only on the level of contamination of soil with those substances, but also on the soil conditions and on the plant species. The findings in this study agree with that of the above researchers, in that, inspite of performance of soybean in respect of high leaf area production and increase in level of Nitrogen and Phosphorus and other parameters measured, maize plant still produced higher yield than soybean and Bambaragroundnut.

This specie response could be attributed to genetical make up of the plants as already mentioned . Also studies have shown that plants reactions to soil contamination with crude oil derivatives vary (Dominquez-Rosado and Pitchel, 2004).

Conclusively, the study revealed that higher dose of diesel oil pollution has negative effect on agricultural crops thereby limiting food production in affected areas causing diseases and hunger, therefore Government agencies and multinational companies should maintain a consistence effort in reducing petroleum products spill in agricultural lands.

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