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Full Length Research Paper

Effect of gasoline diesel fuel mixture on the germination and the growth of *Vigna unguiculata* (Cowpea)

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The effects of gasoline fuel/diesel mixture on the germination of seeds of *Vigna unguiculata*, the survival of the seedlings and the growth of the plant were evaluated in this study. It involved adding 10, 20, 30, 40 and 50 ml of mixture of equal proportions of the two petroleum products to 5000 g of soil and sowing seeds of the *V. unguiculata* in the soils after which the survival and the growth of the plants were evaluated. Although the percentage germination of the seeds of the plant decreased as the quantity of the mixture added to the soil increased only the 40 and 50 ml treatments led to significant reduction of the percentage germination of the seeds ($p < 0.05$). The seedling survival decreased as the volume of the mixture added to the soil increased with no seedling surviving in the soil treated with 50 ml mixture of diesel and gasoline fuels 38 days after the germination of the seeds. The shoot length of the plant was adversely affected by the mixture of the petroleum products especially at week 9 of growth ($p < 0.001$). The dry weight of the plants grown in the soils treated with gasoline/diesel fuel mixture increased as the quantity of the mixture added to the soil increased with the 40 and 50 ml treatments having significant effects on the dry matter content and the root length of the plants ($p < 0.001$). All the treatments significantly inhibited the leaf area development of the plant at week 9 ($p <0.001$). Significant differences ($p < 0.05$, 0.01 and 0.001) were observed among the plants in the different treatments for the different parameters measured. The results obtained in this study showed that spillage of mixture of gasoline and diesel poses threats to the survival and development of plants.

Key words: Gasoline oil, diesel oil, *Vigna unguiculata*, germination, survival

INTRODUCTION

Pollution of the soil with petroleum derivatives is often observed in municipal soils around industrial plants and in areas where petroleum and natural gas are obtained (Adam et al., 2002; Clark, 2003). Processing and distribution of petroleum hydrocarbons (Ayotamuno et al., 2006) as well as the use of petroleum products leads to contamination of soil. Changes in soil properties due to contamination with petroleum-derived substances can lead to water and oxygen deficits as well as to shortage of available forms of nitrogen and phosphorus (Wyszkowska and Kucharski, 2000). Contamination of the soil environment can also limit its protective function, upset metabolic activity, unfavourably affect its chemical characteristics, reduce fertility and negatively influence plant production (Gong et al., 1996; Wyszkowski et al., 2004; Wyszkowski and Wyszkowska, 2005). This threatens human health and that of the organisms that are dependent on the soil (Aboribo, 2001).

The increasing use of diesel oil in diesel engines of cars, industrial trucks and generators has led to an increased demand for diesel oil (Ogbo, 2009) and accidental spillage of diesel and pollution of agricultural lands. Diesel oil is one of the major products of crude oil and it constitutes a major source of pollution to the environment (Nwaogu et al., 2008). 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 (Hill and Moxey, 1960). Soil pollution through such many small and common sources of these products poses large environmental threat (Wyszkowski and Ziolkowska,

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2008). Diesel spills on agricultural land generally reduce plant growth (Nwaogu et al., 2008). Diesel oil reduces soil fertility and soil microflora population (Torstenssen et al., 1998). Wyszokowski and Ziolkowska (2008) also reported that the addition of diesel oil to the soil led to a significant reduction of organic carbon content of the soil. Diesel oil is phytotoxic to plants at relatively low concen-trations. Ogbo (2009) reported that diesel oil contami-nation of the soil caused a reduction in the length of the radicles of *Arachis hypogea, Vigna unguiculata, Sorghum bicolor* and *Zea mays.* The findings of Anoliefo et al. (2001) show that soil from abandoned mechanic village in Nigeria depressed and inhibited the growth *A. hypogea*.

Gasoline is a complex mixture of organic compounds and it also has been shown to be toxic to plants (Anon, 2003; Trapp et al., 2001).Treatment of soils with crude oil, automotive gasoline oil (AGO) and spent engine oil significantly delayed the period of germination, reduced percentage germination, plant height, leaf production and biomass of *V. unguiculata* (Adedokun and Ataga, 2007). Addition of petrol to the soil in quantities above 2.5 cm^3/kg of soil leads to decreased potassium levels in the soil (Wyszkowski and Ziolkowska, 2008).

Although the effects of the individual petroleum products on plants have been evaluated by many studies (Siddiqui and Adams, 2002; Anon, 2003; Andrade et al., 2004; Adedokun and Ataga 2007; Shahidi, 2007), there is the need to study the combined effects of some of the petroleum products on plants. This is because most of them are either spilled or discharged into the environment at the same time or on same environment at different times. This manner of discharge occurs mainly on soil and in water bodies around mechanic villages. This makes it difficult to point to one particular petroleum product as the cause of a particular effect on plants. This study therefore evaluates the effects of gasoline/diesel fuel mixture on the germination, survival, growth and performance of *V. unguiculata*. Information obtained from this study will help to give a clearer idea of what happens to plants grown in soil polluted with a mixture of petroleum products.

METHODOLOGY

This study was conducted in a screen house in the Botanical garden of the University of Lagos using thirty paint buckets of equal diameter (24 cm in diameter) and each filled up to ¾ height with loam soil. Each bucket was polluted with a known volume of gasoline/diesel fuel mixture in equal proportions. The treatment volumes of the mixture added to the soil were 10, 20, 30, 40 and 50ml and each volume was added into five buckets. The control had no gasoline/diesel mixture and also had five buckets. Ten seeds of the *V. unguiculata* (IT845-2246-4 accession) were sown into each bucket and the percentage germination from each treatment calculated after ten days of sowing as described by Njoku et al. (2008).

The buckets were later grouped into two groups: (1) two buckets of each treatment used for survival studies (2) three buckets of each treatment used for growth and performance studies. The percentage survival of the seedlings in the different treatments was calculated as described by Njoku et al (2008) 38 days after sowing. The growth and performance was recorded at weekly intervals after the germination studies for 28 days using plant samples obtained by carefully uprooting one plant from each bucket. The shoot length, the dry matter content, leaf area, leaf area ratio and relative growth rate of the plants were determined at each time. The shoot length was determined as described by Njoku et al. (2008). The dry matter content was determined after oven drying the plant sample at 60°C for 24 h (Merkl et al., 2004). The leaf area of the plant was calculated using the formula: 0.5LB (L = length and B = breadth) after measuring the length and the breadth of a leaf of each plant sample according to Pearcy et al. (1989). The leaf area ratio and relative growth rate were calculated using the formulae of Kang and van Iersel (2004) and McGraw and Garbutt (1990) respectively as follows:

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

$$\text{Relatively growth rate} = \frac{(\ln w_2 - \ln W_1)}{t_2 - t_1}$$

The data obtained were statistically analyzed with ANOVA followed by Bonferonni posttests at 5, 1, and 0.1% levels of significance using the Graph pad Prism 4.0 software.

RESULTS

The percentage germination of the seeds and the survival of the seedlings were affected by the quantity of gasoline/diesel mixture added to the soil. The survival generally decreased with the increase in the quantity of gasoline/diesel mixture in the soil while the percentage germination of the seeds decreased slightly with increased gasoline diesel mixture in the soil (Figures 1 and 2 respectively). Both the 40 and 50 ml treatments a significant reduction on the percentage germination of the seeds ($P < 0.05$).

Tables 1 and 2, respectively indicates the effect of gasoline/diesel oil mixture on the shoot and root length of *V. unguiculata.* The shoot length and the root length of *Vigna unguiculata* were inversely proportional to the gasoline/diesel oil mixture added to the soil. However, the shoot length and the root length of the plant were only significantly affected by the 20, 30, 40 and 50 ml gasoline/diesel fuels mixture treatments ($p < 0.05$, 0.01 and 0.001).

The treatment of the soil with gasoline/diesel oil mixture significantly affected the dry matter content of *V. unguiculata* ($p< 0.05$, 0.01, and 0.001). The dry matter content of the plant generally had a linear relationship with the quantity of gasoline/diesel fuel mixture added to the soil (Table 3). However, the dry weight of the plant grown in soil polluted with 50 ml of gasoline/diesel oil mixture was generally lower than that of the plant grown in soil treated with 40 ml of the mixture.

The leaf area of the plants generally decreased as the quantity of gasoline/diesel mixture added to the soil increased (Table 4). At 5, 1 and 0.1% levels of signifi-

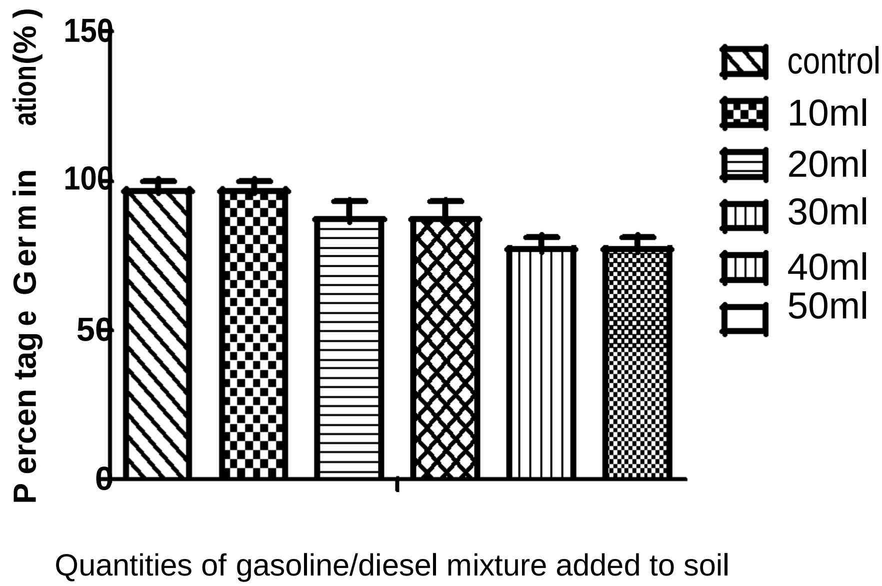

Figure 1. The effect of gasoline/diesel oil mixture on the germination of cowpea seeds.

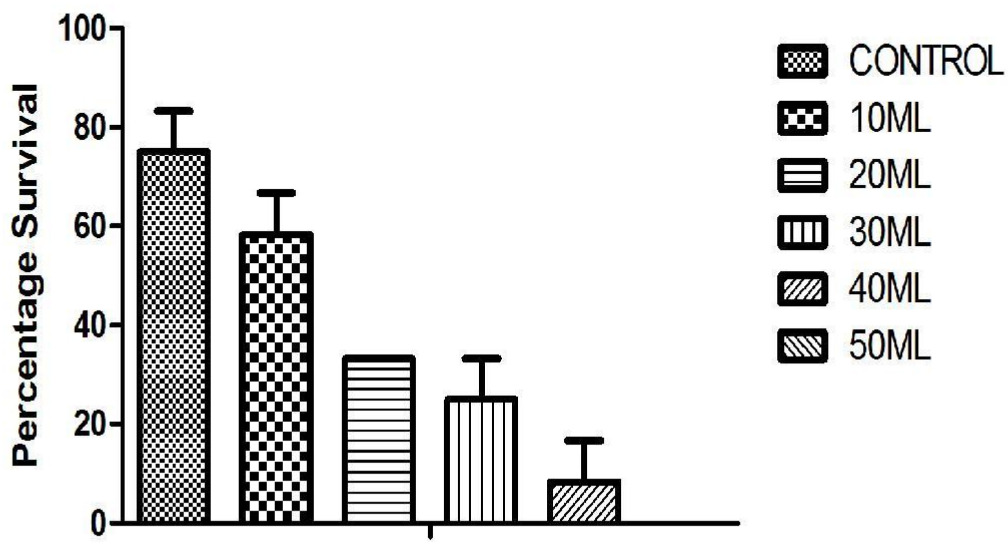

Figure 2. The effect of gasoline/diesel oil mixture on the survival of cowpea seedlings. The value are means ± standard error of means of three replicate values.

Table 1. The shoot length (cm) of cowpea grown in gasoline/diesel oil mixture polluted soil.

		10 ml	20 ml	30 ml	40 ml	50 ml
Week 1	37.00±4.73	32.33±1.76	31.17±2.68	27.43±1.72	23.17±0.44*	17.17±1.58***
Week 2	51.67±2.41	41.33±1.45	42.73±1.88	37.23±1.14**	30.33±0.52***	19.97±0.63***
Week 3	64.67±5.04	57.67±2.85	56.50±0.87	47.00±3.51**	31.83±0.60***	22.00±0.53***
Week 4	73.67±8.41	68.67±5.78	56.67±2.03**	50.02±5.55***	38.60±1.00***	16.67±2.40***

Note: The value are means ± standard error of means of three replicate value, * means significantly different from control ($p < 0.05$), **means significantly different from control($p < 0.01$), ***means significantly different control ($p < 0.001$).

Table 2. The root length (cm) of cowpea grown in gasoline/diesel oil mixture polluted soil.

		10 ml	20 ml	30 ml	40 ml	50 ml
Week1	3.43±0.23	2.77±0.15	2.63±0.13*	1.97±0.15***	1.73±0.18***	1.33±0.18***
Week 2	3.53±0.23	2.83±0.23	2.83±0.09	2.03±0.03***	1.97±0.09***	1.83±0.07***
Week 3	3.20±0.20	2.83±0.09	3.27±0.26	3.03±0.18	1.90±0.06***	1.77±0.15***
Week4	3.07±0.23	3.20±0.29	2.77±0.29	2.00±0.06**	2.57±0.52	1.03±0.09***

Note: The value are means ± standard error of means of three replicate value, * means significantly different from control ($p < 0.05$), **means significantly different from control ($p < 0.01$), ***means significantly different from control ($p < 0.001$).

Table 3. The dry matter content (g) of cowpea grown in gasoline/diesel oil mixture polluted soil.

		10 ml	20 ml	30 ml	40 ml	50 ml
Week 1	0.04±0.01	0.08±0.01	0.11±0.01**	0.11±0.02**	0.18±0.00***	0.15±0.03***
Week 2	0.06±0.00	0.07±0.01	0.12±0.01*	0.12 ±0.01*	0.18±0.01***	0.17±0.00***
Week 3	0.08±0.02	0.11±0.01	0.12±0.01	0.14±0.01**	0.18±0.00***	0.18±0.01***
Week 4	0.12±0.02	0.13±0.01	0.13±0.00	0.14±0.02	0.22±0.02***	0.28±0.03***

Note: The value are means ± standard error of means of three replicate value, * means significantly different from control ($p < 0.05$), **means significantly different from control ($p < 0.01$), ***means significantly different from control ($p < 0.001$).

Table 4. The leaf area (cm^2) of cowpea grown in gasoline/diesel oil mixture polluted soil.

		10 ml	20 ml	30 ml	40 ml	50 ml
Week 1	4.22±0.98	3.04±0.34	2.34±0.24	2.34±0.34	1.63±0.34**	1.12±0.30***
Week 2	4.79±1.14	3.23±0.44	3.03±0.09	3.22±0.17	2.52±0.22*	1.42±0.17***
Week 3	5.93±0.82	5.33±0.38	4.66±0.31a	3.54±0.14**	3.20±0.16**	1.61±0.20***
Week 4	7.94±1.42	4.87±0.58***	4.09±0.10***	3.95±0.03***	3.52±0.08***	1.13±0.19***

Note: The value are means ± standard error of means of three replicate value, * means significantly different from control ($p < 0.05$), **means significantly different from control ($p < 0.01$), ***means significantly different from control ($p < 0.001$).

Table 5. The leaf area ratio (cm^2/g) of cowpea grown in gasoline/diesel oil mixture polluted soil/.

		10 ml	20 ml	30 ml	40 ml	50 ml
week 1	121.03±31.38	46.67±8.45***	21.93±4.06***	21.52±1.11***	11.77±1.40***	8.56±2.90***
week 2	76.63±20.35	45.73±2.52	25.45±1.85***	25.66±4.45***	14.09±1.67***	6.20±2.34***
week 3	80.77±7.79	50.27±4.63	37.91±2.23**	25.04±2.11***	18.11±0.55***	9.10±0.77***
week 4	64.94±12.42	40.65±4.73	30.77±1.46*	23.25±6.95**	16.02±1.52**	4.10±0.66***

Note: The value are means ± standard error of means of three replicate value, * means significantly different from control ($p < 0.05$), **means significantly different from control different ($p < 0.01$), ***means significantly different from control ($p < 0.001$).

cance, the leaf area of the plants grown in soil with different quantities of gasoline/diesel mixture differed from each other and also from the leaf area of *V. unguiculata* grown in uncontaminated soil. The leaf area ratio of the plant grown in soil polluted with different quantities of gasoline/diesel mixture (Table 5). The leaf area ratio of the plant in the different treatments significantly differed from each other ($p <0.5$, 0.01 and 0.001). The treatment of the soils led to significant reduction in the leaf area ratio of *V. unguiculata*. The relative growth rate of the plants has an inverse relationship with the quantity of gasoline/diesel mixture added to the soil (Figure 3). However the relative growth ratio of *V. unguiculata* grown in soil polluted with 50 ml gasoline/diesel oil mixture was best among the treated soils. The relative growth rate of the plants treated with

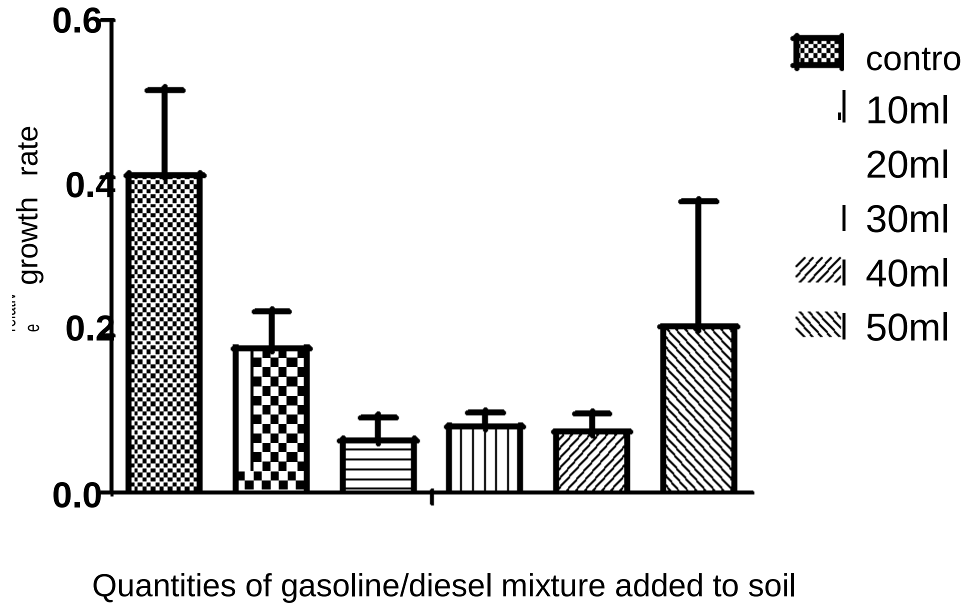

Figure 3. The effect of gasoline/diesel oil mixture on the relative growth rate of cowpea. The values are means ± standard error of means of three replicate values.

20, 30 and 30 ml of the gasoline/diesel fuel were significantly lower than the relative growth rate of the plant grown in uncontaminated soil.

DISCUSSION

Gasoline diesel fuel mixture like the other petroleum products adversely affects the growth and performance of plants as indicated in the results. The effect of the mixtures on the shoot length of *V. unguiculata* observed here were similar to those reported on the effect of spent oil on *Amaranthus hybridus* (Odjegba and Sadiq, 2002). Njoku et al. (2008) also found similar findings on the effect of crude oil the growth of accessions of *Glycine max* and *Lycopersicon esculentum*. Adedokun and Ataga (2007) also showed that treatment of soils with crude oil, automotive gasoline oil and spent engine oil significantly affected the time of germination, percentage germination, plant height, leaf production and biomass of *V. unguiculata* delaying germination and growth rate. Sun et al. (2004) made similar observation when they studied the effect of diesel fuel on the growth of *Nerlum oleander*, beach naupaka, false sandalwood, common ironwood, kou, milo and kiawe.

The decrease in leaf area, leaf area ratio and relative growth rate of *V. unguiculata* in this study due to gasoline/diesel mixture pollution of soil indicates that the mixture interrupts with the growth of the plant. According to Kathirvelan and Kalaiselvan (2007) the leaf surface area determines in large part the amount of carbon gained through photosynthesis and the amount of water lost through transpiration and ultimately the crop yield. Therefore the reduction of the leaf area and the leaf area ratio as was observed in this study implies that there would be low a photosynthetic efficiency of the plant as much of the solar energy emitted by sun would not be absorbed by plant for photosynthesis. This can lead to low yield of the plant with subsequent low availability of food and poor economy due to low sales of such plant's products. Since the relative growth rate measures the efficiency of seedling (Brand, 1991), the reduction in the relative growth rate of *V. unguiculata* we reported in this study simply means that petroleum mixture reduces the efficiency of the plant.

According to Walker et al. (2001), availability of nitrogen in the soil directly affects the relative growth rate of plants. Since petroleum-products are known to reduce nitrogen availability (Agbogidi et al., 2007), that could be the cause of reduction of the relative growth rate of plants by petroleum products. Also since plants grow slower under low nutrient levels Grotkopp and Rejmanek (2007), the reduced relative growth rate observed in this study can be attributed to the low nutrient level in the polluted soil which had been reported by some researchers

According to Wyszkowski and Zoilkowska (2008), proper growth of cultivated plants is dependent on the content of nutrients in the soil. The inhibition of the growth of *V. unguiculata* we observed in this study therefore possibly occurred due to the effect petroleum oil has on soil. The adverse effects could be due to disruption of the absorption and uptake of nutrients by petroleum products (Njoku, 2008). The degrading effect of petroleum – derived compounds on soil leads to severe nitrogen and phosphorus depletion, depletion of water balance and biological equilibrium (Baran et al., 2002).

Dimitrow and Markow (2000) showed that the presence of oil in the soil significantly decreased the available forms of phosphorus and potassium to plants. These nutrients (nitrogen, phosphorus, potassium and oxygen) are essential to plat growth and development hence reduction in their bioavailability will lead reduced plant growth.

From our findings it can be concluded that both gasoline and diesel fuels can contaminate soil which may result in low soil fertility. Therefore, it is recommended that mechanic workshops be cited in locations away from farmlands so that with diesel oil and gasoline to such soil will minimized. This will reduce the poor yield and high mortality of plants due to petroleum pollution. The response of cowpea to gasoline/diesel oil mixture indicates that the plant can be used to monitor polluted sites.

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