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Screening of *Abelmoschus esculentus* L. Moench for tolerance to spent engine oil

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ABSTRACT

Objective: To investigate the effect of different concentrations of spent engine oil on the growth of okra (*Abelmoschus esculentus*) plants.
Methodology and results: 4.5 kg topsoil was mixed with 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0 % (w/v) of spent engine oil (SEO). The control was not mixed with SEO (0%). The seeds were sown on these soils and monitored on alternative days. Parameters monitored were chlorophyll and relative water contents of the plants were analyzed after harvest. SEO contamination adversely affected growth and some physiological parameters, depending on concentration. The growth parameters were reduced by higher concentration of the oil. However, 0.2 and 0.4% SEO did not reduce these parameters. The highest relative water content (78.4%) was found in the control plants, followed by those treated with 0.2% spent oil (51.89%), the least being those treated with 0.6% spent oil (1.71%). Chlorophyll contents of okra plants were also reduced by the spent engine oil treatment, highest being recorded in the control plants (0% SEO) and the least were those exposed to 0.4% SEO. The plants grown on treated soil also showed chlorosis and necrosis symptoms at higher oil concentrations (0.6-3.0%).
Conclusion and application of findings: These results indicate that contamination of soil with spent engine oil at very low concentration (0.2%) has no significant ($P < 0.05$) effect on okra plants growth while the higher concentrations adversely and severely affect plant growth. Indiscriminate spillage of spent engine oil affected okra physiology and growth. Therefore, spent engine oil should be properly disposed.

Key words: Spent engine oil, *A. esculentus,* growth, chlorophyll content, relative water content

INTRODUCTION

Soil is the most valuable component of the farming ecosystem and environmental sustainability largely depends on proper soil maintenance. Sustainable use of this natural resource on which agriculture depends is absolutely necessary for agricultural productivity. Soil pollution by crude oil and petroleum products such as fuel oils, spent engine and diesel fuels are presently a menace in Nigeria, particularly in big cities. Oil pollution in whatever form is toxic to plants and soil micro-organisms (Adenipekun & Kassim, 2003; Adedokun *et al*., 2007).

Disposal of spent engine oil in the big cities in Nigeria has been persistently problematic since many automobile mechanics dispose these oils indiscriminately either in gutters or open lands. This practice adversely affects plants, microbes and aquatic lives (Nwoko *et al*., 2007; Adenipekun *et al*., 2008), because of the large amount of hydrocarbons and highly toxic polycyclic aromatic hydrocarbons contained in the oils (Vwioko & Fashemi, 2005).

Edebiri and Nwaokwale (1981) reported that metals present in spent lubricating oil are not necessarily the same as those present in the unused lubricant. Whisman *et al.* (1974) observed that most heavy metals such as vanadium, lead, aluminium, nickel and iron, which were below detection levels in unused lubricating oil, were present in high levels in used oil. These high amounts of heavy metals in spent crude oil may be one cause of the retarded growth and chlorosis of leaves of plants growing in oil polluted soils (Udo & Fayemi, 1975).

Although the disposal of used engine oil in the environment is common in Nigeria, the impact of this on the production of vegetables including okra has not been ascertained. *Abelmoschus esculentus* (Malvaceae) is an erect, semi-woody herbaceous annual, which is highly variable in growth from less than 1m to over 3m in height. The flowers are yellow and purple and finger-like fruit occur at the leaf axis. It is ubiquitously cultivated in Nigeria. The leaves and fruits of okra plant are eaten fresh and fried as pot-herb. The leafy tops are grazed by stock and game. The fruits are rich in minerals and vitamins while the stem bark yields fibre (Burkill, 2000).

Anoliefo *et al.* (2006) reported that waste pit soil from drilling waste dumps in Kutchalli oil drilling area of Nigeria completely inhibited the germination of maize and bean seeds. Waste pit soil in combinations with different properties of Kutchalli soil resulted in growth (germination, height of plants, number of leaves, leaf area) values that were inferior to the plants grown in uncontaminated soil. It was concluded that waste pit soil was toxic to plant growth and development. Similarly Anoliefo and Edegbai (2006) investigated the effect of spent lubricating oil on the growth of *Solanum melongena* and *S. incanum* and found that the emergence of *S. incanum* was completely inhibited by 4 and 5% oil content in amended soil while height and leaf values parameters of both plants were depressed at oil concentrations above 3% oil amended soil. Adenipekun *et al.* (2008) reported that spent engine oil at all concentrations delayed the germination of *Corchorus olitorius* by 2 days (compared to control) and caused a significant reduction in all the growth parameters in plants grown on contaminated soil compared to control plants.

This study aimed at investigating the effect of spent engine oil on the growth parameters and chlorophyll content of *Abelmoschus esculentus* as indicators of the tolerance level of the vegetable to oil pollution.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of Botany and Microbiology Department, University of Ibadan, Nigeria, based on the method of Odjegba and Sadiq (2002). Topsoil obtained from the nursery of the department was sieved to remove gravel and debris with a 2mm mesh. Seeds of *Abelmoschus esculentus* were obtained from the National Horticultural Research Institute (NIHORT) Ibadan and spent engine oil was obtained from two different auto-mechanics workshops in Ibadan. A sample of 4.5kg of soil was filled into black polythene bags. The bags were arranged factorially in completely randomized design.

The soil was treated with engine oil to achieve 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 and 3.0% v/w according to the method of Adenipekun and Kassim (2006). The mixing was gradually done to ensure thorough and even mixing (untreated soil (0% oil) served as the control). The soils were watered to 60% field capacity. The seeds of *A. esculentus* were broadcast on the soil surface and covered with the soil. Watering was done on every alternate day. The plants were harvested six weeks after planting by cutting the polythene bags at the sides with the soil intact so as not to loose any part of the roots. The soil was washed away under running water.

Plant growth measurements: The height of each plant was measured at regular interval of seven days from the soil level to the terminal bud using a meter rule. . The leaves produced were counted every 7 days (Odjegba & Sadiq, 2002). The leaf area was determined by measuring the base the middle and the upper part of leaf with a ruler and calculated using the formula breadth x length x 0.85 and the girth was taken 2cm above the soil level; the same point was maintained till the end of the experiment to ensure accurate reading, using Vernier calipers.

Leaf chlorophyll content: The chlorophyll content of okra leaves was estimated according to Hipkins and Baker (1986). Two grams of the fresh leaves were collected in polyethylene bag. These were ground with pestle and mortal in 80% v/v aqueous acetone in dim light and filtered with No. 1 Whatman filter paper. Ten ml of the filtrate (extract) was put into flat bottom volumetric flask and made to 50ml with 80% v/v aqueous acetone. Ten ml of this mixture was taken into a cuvette and the absorbance (A) was read in a spectrophotometer at 645, 653 and 663nm. The measurements were replicated three times for each treatment. The chlorophyll content (mg/l) in each of the samples was calculated using the following simultaneous equation:

Chlorophyll (chla) = $12.7A_{663} - 2.69A_{645}$

Chlorophyll b (chlb) = $22.9A_{645} - 4.68A_{665}$

Total chlorophyll (T chl) = $20.2A_{645} + 8.02A_{663}$

Leaf Relative Water Content (RWC): Fresh leaf samples were collected from each treatment. A sharp cork borer was used to cut the leaf samples into small discs (5cm diameter). These were weighed and recorded as sample fresh weight (W), after which the samples were hydrated to full turgidity in distilled water for four (4) hours under normal room light and temperature. After 4 hr, the samples were taken out of water and quickly dried of any surface moisture using filter paper, and immediately weighed to obtain full turgid weight (TW). Samples were then oven dried at 80°C for 24 hr and allowed to cool in desiccators, and weighed to determine the dry weight (DW). Relative water content was calculated using the mathematical expression below:

$$\text{RWC (\%)} = \frac{W - DW}{TW - DW} \times 100$$

Where:

W = sample fresh weight

TW = sample turgid weight

DW = Sample dry weight

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and means of treatments further compared using t-test.

RESULTS

The results show marked difference between the plants grown in non-contaminated soil and those grown in soil with spent engine oil. *A. esculentus* planted in soil treated with oil started germinating after 6 days compared to 4 days for plants in untreated soil (control).

Table 1 shows data on the effect of the spent engine oil (SEO) on leaf area of *A. esculentus*.There was no significant difference ($P < 0.05$) between the control plants and those grown in soil with 0.2% oil. The leaf area decreased as the oil concentration increased above 0.4% but at the third week, there was no significant difference between the leaf area of plants exposed to between 0.6 – 3.0% SEO. All the plants grown in soil treated with 0.6 -3.0% SEO died before week 5 after planting.

Table 1: Effect of spent engine oil concentration on leaf area (cm^2) of *Abelmoschus esculentus*.

Engine oil content (%)	Leaf area (cm^2)	
	3rd week	5th week
0l	97.4^a	49.1^a
0.2	97.4^a	48.7^a
0.4	69.3^b	24.0^b
0.6	3.77^c	0.00
0.8	2.45^c	0.00
1.0	2.35^c	0.00
2.0	2.2^c	0.00
3.0	1.3^c	0.00

Each value is a mean of 3 replicates. Mean values followed by same letters along the columns are not significantly different at $P \leq 0.05$.

Table 2 shows data on the effect of different concentrations of spent engine oil on stem girth of *A. esculentus.* Spent oil above 0.6% significantly reduced the girth of the plants from the 4th week. In the 4th, 5th and 6th weeks the girths were similar in the plants grown on 0.2 and 0.4% contaminations.

The relative water content (RWC) of the plants decreased with increasing concentration of spent oil

(Table 3). The plants were harvested after the 4th week after planting to determine their RWC. The RWC of the control plants were significantly higher than those grown in soils contaminated with SEO. However, those grown in 0.2% oil had higher RWC than those grown in 0.4 – 0.6%. The relative water contents of the plants in 0.4 and 0.6% were not significantly different (Table 3).

Table 2: Effect of spent engine oil concentration on stem girth of *Abelmoschus esculentus.*

Time	Oil concentration (%)							
	Control (0)	0.2	0.4	0.6	0.8	1.0	2.0	3.0
4th Week	0.33a	0.27b	0.25b	0.14c	0.13c	0.12c	0.11c	0.10c
5th Week	0.40a	0.36b	0.30b	0.00	0.00	0.00	0.00	0.00
6th Week	0.43a	0.41b	0.33b	0.00	0.00	0.00	0.00	0.00

Each value is a mean of 3 replicates. Means with the same letter in the same row are not significantly different at P ≤ 0.05.

Table 3: Effect of spent engine oil concentration on the relative water content of *Abelmoschus esculentus* at 6th weeks after planting.

Spent oil concentration (%)	Relative water content
Control	78.4b
0.2	51.89b
0.4	3.42c
0.6	1.71c

Fig. 1 shows the effect of different concentrations of spent engine oil on the number of leaves of *A. esculentus.* After two weeks of germination, the control plant had four leaves; the plants in soil treated at 0.2% oil concentration had 3 leaves while those in soil at 3.0% oil had 2 leaves. At four weeks after germination, the number of leaves on control plants and those in soil with 0.2% oil treatment increased to 5 while the leaves on plants grown in soil with 0.6 – 3.0% spent engine oil shed their leaves before the end of the experiment. The height of *A. esculentus* was affected by different SEO concentrations (Fig. 2). After two weeks of germination, mean plant height was 7.4cm in the control plants and 6.8cm in soil treated with 0.2% SEO. Plant height was 5.3cm in soil with 3.0% engine oil. At the end of six weeks the height of control plants increased to 26.2cm while that of plants in 0.2% engine oil contaminated soil was 25.0cm. Plants from 0.8% - 3.0% developed necrosis and dried off. This shows that plant height decreased with increase in concentration of engine oil in the soil. As shown in Fig. 3, the highest chlorophyll content was recorded in okra plants grown in uncontaminated soil (16mg/l) while the lowest was recorded in soil with 0.4% engine oil (13.5mg/l). Plants grown in soils with higher oil concentrations died after the 4th week; therefore their chlorophyll contents could not be determined. The effect of SEO on *A. esculentus* was significant on its leaf chlorophyll content.

DISCUSSION

Germination of Okra plants was delayed in the soils contaminated with spent engine oil, though this effect did not vary with oil concentration since almost all the seeds in all the contaminated soils germinated on the same day. The presence of SEO in the soil-plant microenvironment appears to have affected normal soil chemistry wherein nutrient release and uptake as well as amount of water have been reduced (Nwoko *et al.* 2007). Atuanya (1987) reported that seeds of *C .odorata* failed to germinate even after 30 days of sowing.

The decrease in height of plant with increase in concentration of SEO is probably due to the non-availability of adequate water, which possibly affected the nutrient uptake and mobility. Nevertheless, the growth of the plants was not significantly affected at lower SEO concentrations. This implies that *A. esculentus* can tolerate low levels of SEO contaminations. This is reflected in the number of leaves, leaf area production and the stem girth of the plants in low SEO contamination, which were similar to those of the plants grown in uncontaminated soil.

The poor growth recorded in soils contaminated with higher amounts of SEO was correlated to the level of the relative water contents (RWC) recorded. The RWCs of the plants grown in SEO contaminated soils were highly reduced. This translated into low metabolic processes in those plants that affected their growth performance.

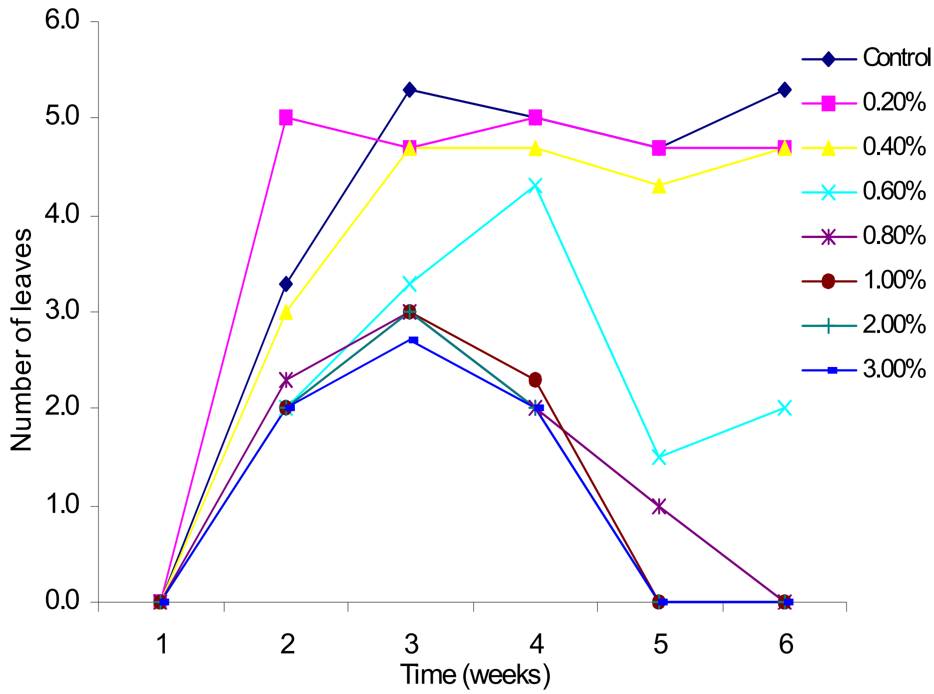

Figure 1: Effect of different concentrations of spent engine oil on the number of leaves of *A. esculentus*.

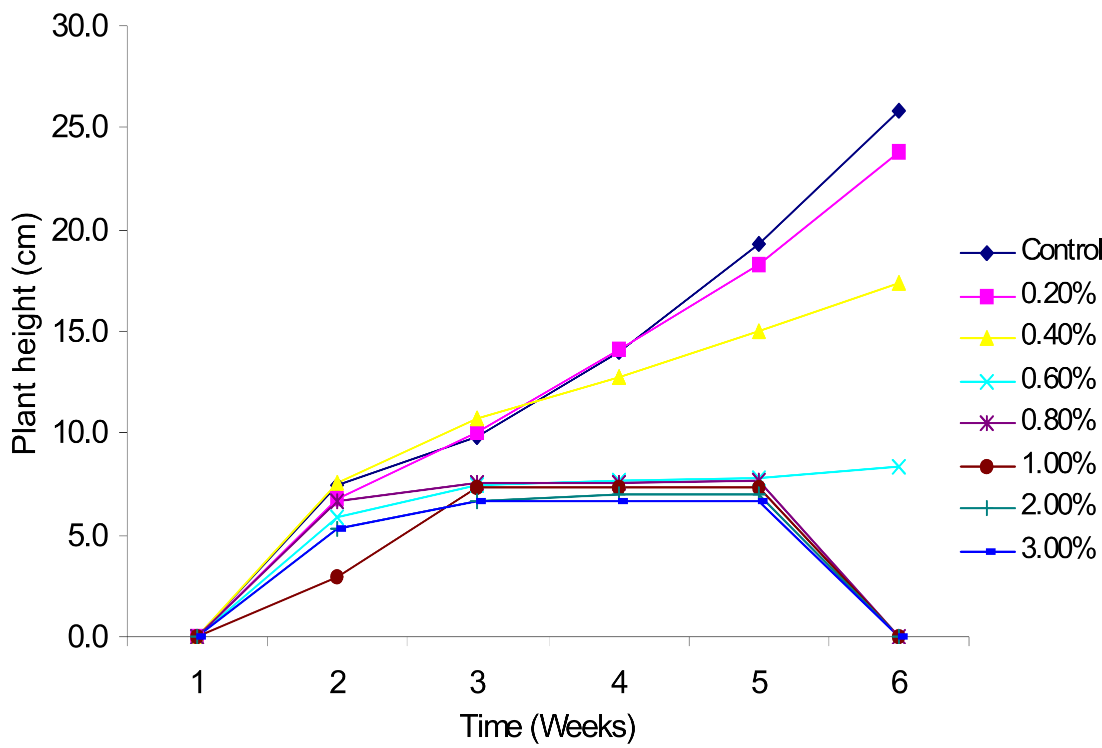

Figure 2: Effect of different concentrations of spent engine oil on the height of *A. esculentus.*

Gill *et al.* (2005) made similar observations on *Chromolaena odorata* grown in soil treated with crude oil. The SEO treatment had adverse effect on chlorophyll synthesis. The decrease in chlorophyll level with increase in SEO concentrations was similar to the findings of Odjegba and Sadiq (2003) where reduction in chlorophyll and protein levels were reported in *Amaranthus hybridus* grown in soil contaminated with engine oil.

Reduced chlorophyll production might be connected with the low level of RWC recorded in SEO treated plants and less optimal functioning of the roots due to low availability of water and oxygen in those soils. The plants were unable to survive in soils treated with high levels of SEO and they died after the fourth week. Before the death of the plants there was stagnation of growth after the third week. The investigation revealed dead roots of the plants, which corroborates the findings of Gill *et al.* (2005).

This investigation shows that spent engine oil pollution has potential adverse effects on Okra plants, though plants can tolerate low levels of contamination (<0.2%). Hence, appropriate disposal of spent engine oil should be taken seriously by those handling it as well as regulatory institutions.

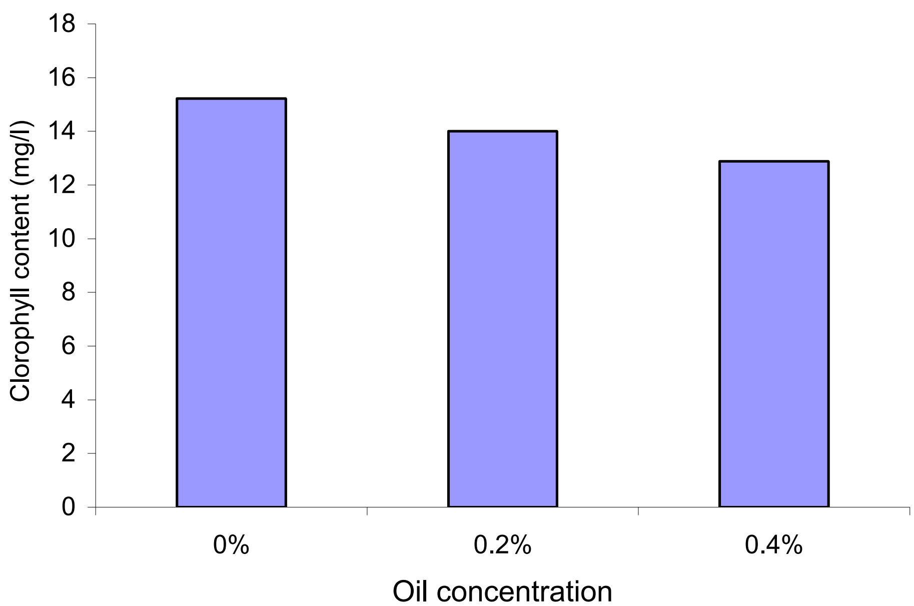

Figure 3: Chlorophyll content of *Abelmoschus esculentus* (Okra plants) after 6 weeks of growth in soil contaminated with spent engine oil in soil.

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