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Full Length Research Paper

The effect of crude oil on microorganisms and dry matter of fluted pumpkin (*Telfairia occidentalis*)

M. A. Ekpo* and C. J. Ebeagwu

Department of Microbiology, University of Uyo, Uyo, Nigeria.

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The effect of crude oil on microorganisms and dry matter of fluted pumpkin (*Telfairia occidentalis*) was investigated. The soil was treated with different levels of crude oil: 0% (control), 1, 3 and 6% v/w. There was no significant different in bacteria count after 35 days in all the treatments. The mean fungi count of 4.5±0.4 x10^4 and 3.3±0.5x10^4 cfu/g in the 3 and 6% pollutions, respectively. Pumpkin seeds had 100% emergence/germination in both the control and 1% oil treated soils while the 3 and 6% treatments recorded 85 and 33% respectively. The 3% and the 6% crude oil treated soils significantly (P>0.05) reduced the dry matter of leaf, stem and root of *T. occidentalis.* The pollution levels of 3% and above was noted to be harmful to the germination and growth of the plant.

Key words: Pumpkin (*Telfairia occidentalis*), crude oil, microorganism, emergence, germination.

INTRODUCTION

Crude oil, a complex mixture of hydrocarbon, liquid in their natural state are classified into aliphatic, alicyclic and aromatic compounds (Atlas and Bartha, 1973). Most of these components are known to be toxic in nature to different biomass (Walker and Colwell, 1976), and this has raised considerable concern on the subject of crude oil pollution especially on aerable agricultural land. Though oil spillage is a widespread phenomenon, it is comperatively more frequent in the developing countries than in the technologically developed nations.

In Nigeria, a large amount of crude oil is spilled annually into the environment. There was about 2,000 oil spillage in Nigeria between 1976 and 1988 which involved about 2x10^6 barrels of crude oil into the environment. Oil spillage have been known to exhibit various deleterious effects on both plants and microorganisms. Crude oil spillage on soil generally retard plant growth (Gill and Sandota, 1976, Glouse et al., 1980, Atuanya, 1987, Ekpo and Nwankpa, 2005), reduces aeration by blocking air spaces between soil particles hence create condition of anaerobiosis (Rowell, 1977) and causes root stress in plant which also reduces leaf growth (Smith et al., 1989).

The initial reaction of the micro organisms as it gets in contact with oil in the soil is a reduction of activity due to reduced air availability. This has been noted to arise from selective destruction of aerobic bacteria and fungi thus leaving the resistant and adaptive microbial strains to proliferate (Odu, 1981). However microorganisms remain the only organ of effective natural removal of crude oil in a contaminated soil. This ability however depends on a number of factors which include temperature, viscosity of the oil, coarseness of the soil and the level of oil in the environment. It has been reported that in tropical conditions, crude oil disappears with unprecedented rapidity in freely well-drained soils but degradation is slowed down by poor aeration (Odu, 1981). Roscoe et al. (1989) have also reported the increase in anaerobic microorganisms in crude oil polluted soil.

A number of aerobic microorganisms have been isolated from oil polluted soil. They include actinomyces, fungi, heterotrophic bacteria and phototrophic microorganisms. Okpokwasili and Okorie (1988) observed that microorganisms may either be involved in the crude oil degradation or be present as inert passengers or probably metabolizing the intermediate products of pollutant-oxidizing organisms. Common among heterotrophic bacteria genera that have been isolated form petroleum–polluted soil are *Streptomyces, Bacillus, Micrococcus, Arthrobacter, Flavobacterium, Methano-bacterium Achromobater, Cloctridium, Thiobacillus, pseudomouas, Alcaligenes* and filamentous fungi as *Penicillium, Aspergillus, Fusarium Cladosparium* and *Candida* (Antai and Mabomo, 1989)

Telfairia occidentalis is a vegetative crop belonging to

*Corresponding author. E-mail: emacopron@yahoo.com.

the family Cucurbitaceae and is grown in South Eastern Nigeria. It is a perennial woody timber grown for its leaves and seeds, which are very nutritious (Odoemena, 1991). The stem is puberulous and cylindrical at maturity with tendrils which are branched and spirally coiled. The male inflorescence is a raceme while the female flower is solitary. The female plants have longer vegetative growth and development and bears the pods that contain the highly nutritious seeds.

Analysis of the pumpkin leaf shows that it contains nitrogen (6%), protain (37%), phosphorus (0.6%), potassium (0.4%) and manganese (180 ppm) while the seed on the other hand has been shown to contain protein 30%), fat (50 g), carbohydrate (10 g), fibre (2 g), calcium (40 mg), iron (10 mg), vitamin A (0.l mg), thiamine (0.2 mg), an nicotinamide (2 mg) (Tindall, 1983). After a major oil spillage in the Niger Delta, it was observed that the main crops attacked were tubers and root crops such as yam (*Dioscors* sp), cassava (*Manihot esculenta*), cocoyam (*Colocasia* and *Xanthosama* spp.), while the vegetable include peppers (*Capsicum fruitenscen*) pumpkin (*T. occidentalis*) sugar cane (*Sacchariaum commonsus*) (Odu, 1972).

This research was therefore carried out to evaluate the effect of different concentrations of crude oil on the soil microorganisms, germination and dry matter production of *T. Occidentalis*

MATERIALS AND METHODS

Soil analysis

The garden soil used for the planting was analysed before the pollution with crude oil. Composite soil samples from the plot were bulked together in the laboratory, crushed to break the large soil aggregates and air dried under room temperature 28±2°C for two weeks. The samples were sieved through a 2 mm sieve to remove large particles, debris and stones. For organic matter determination, the sieved soil was ground to pass through a 0.5 mm sieve, from it organic carbon was determined by the dichromate-oxidation method of Walker and Black (1943). Mechanical analysis was done by the hydrometer method using sodium hexametaphosphate as a dispersing agent. Soil pH was determined using a 1:1 soil:water slurry and measured in a Beckman Zerometric pH meter.

The total nitrogen was determined by the micro-kjeldahl digestive method and the content in the digest measured calorimetrically (Odu et al., 1986). Available-P in the soil sample was determined by the Bray's P method. Exchangable Ca, Na, Mg and K were extracted with 1N neutral ammonium acetate solution. Thereafter K, Na and Ca were determined by flame photometer while the Mg was read from Atomic Absorption Spectrometer (AAS). Effective cation exchange capacity (ECEC) was calculated by the summation of all exchangeable bases and acids.

Soil preparation and planting

Twenty-four bags containing 700 grams each of garden soil samples collected from the University of Uyo Teaching and Research farm were arranged in four sets. The soil belongs to the ultisol series of the USDA soil Taxonomic system of classification. Each set made up of 6 bags were treated with different levels of crude oil. Group one had no crude oil and served as control. Seven milliliters of crude oil was introduced into each of the 6 bags in group two and that represented 1% pollution. The 3 and 6% pollution for groups three and four were made by introducing 21 and 42 ml of crude oil into each bag of soil respectively. The crude oil was properly mixed in each bag and labelled. They bags were then laid out in a completely randomized design, in the field.

Three pumpkin seeds were planted in each bag and after the two seed leaf stage they were thinned down to two plants. The emergence/germination were recorded in all the set ups

Microbial analysis

The soil samples were collected from the first day of pollution. Ten grams from each treatment was taken and serial dilution was carried out. From the fifth dilution, with a pipette 1 ml was taken for isolation of microorganisms using the pour plate method of Collins and Lyne (1976). This process was repeated at 7 days interval for five times. The microbial load was determine at each time and different microbial species were isolated based on their colony morphology, characterized and identified based on the method of Cowan (1985) and Fawole and Oso (1988).

Dry matter analysis

Dry matter analysis was done by destructive sampling method of Ekpo and Odu (2000). Two plants were uprooted randomly from each treatment and was partitioned into the root, the stem and the leaves. The fresh weight was taken and recorded as "a" and they were subsequently dried in the hot air oven for 12 h at 180°C to a constant weight. The final dry weight was recorded as "b". This was repeated at 7 days interval for four times. Percentage dry weight was calculated using the formula:

Dry wt (%) = (b/a) x 100

Where a = fresh weight of plant part and b = dry weight of plant part.

The moisture content was determined by subtracting the dry weight from the fresh weight.

RESULTS

Germination/emergence

The emergence/germination of pumpkin seeds in soil treated with different concentrations of crude oil is presented in Table 1. It was observed that the control and the 1% crude oil treated soils had 100% germination while the 3% and 6% had 85% and 33% germination, respectively. The control and the 1% treatments revealed that more that 50% of the seeds emerged within seven days. The 6% polluted soil had delayed emergence till the tenth day after planting.

Microbial count

The bacterial and fungal from soil treated with different concentrations of crude oil are shown in Tables 2 and 3.

Table 1. Emergence of pumpkin seeds in soil treated with different concentrations of crude oil.

Conc. (%)	Emergence/germination								Total Germination	Germination (%)
	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 15		
0	2	2	0	2	0	0	0	0	6	100
1	2	0	1	1	1	1	0	0	6	100
3	0	1	0	0	2	2	0	0	5	83
6	0	0	0	0	0	1	0	1	2	33

Table 2. Bacterial count (cfu/g) from soil treated with different concentrations of crude oil.

Sampling (days)	Crude oil concentration			
	0%	1%	3%	6%
0	1.2×10^6	1.3×10^6	1.2×10^6	1.1×10^6
7	2.4×10^6	1.4×10^6	1.2×10^6	1.2×10^6
14	1.1×10^6	2.2×10^6	1.3×10^6	1.7×10^6
21	4.0×10^6	8.4×10^6	2.7×10^6	2.6×10^6
28	3.0×10^6	2.3×10^6	2.9×10^6	5.5×10^6
35	3.5×10^6	3.0×10^6	2.1×10^6	2.6×10^6
Mean	$2.5 \pm 0.2 \times 10^6$	$3.1 \pm 0.4 \times 10^6$	$1.9 \pm 0.1 \times 10^6$	$2.4 \pm 0.3 \times 10^6$

Standard error of mean (Sx) = 3.3×10^5; $LSD_{0.05} = 0.85 \times 10^6$.

Table 3. Fungi count (cfu/g) from soil treated with different concentrations of crude oil.

Sampling (days)	Crude oil concentration			
	0%	1%	3%	6%
0	2.4×10^4	1.4×10^4	1.3×10^4	1.2×10^4
7	9.0×10^4	3.4×10^4	4.7×10^4	5.6×10^4
14	2.4×10^4	6.0×10^4	7.0×10^4	8.0×10^4
21	1.0×10^4	1.6×10^4	5.0×10^4	3.0×10^4
28	3.4×10^4	1.5×10^4	4.0×10^4	4.2×10^4
35	1.8×10^4	2.0×10^4	5.0×10^4	5.1×10^4
Mean	$3.3 \pm 0.5 \times 10^4$	$2.6 \pm 0.3 \times 10^4$	$4.5 \pm 0.3 \times 10^4$ *	$4.5 \pm 0.4 \times 10^4$ *

Standard error of mean (Sx) = 4.5×10^3; $LSD_{0.05} = 0.85 \times 10^4$.
*Significantly higher (P>0.05).

The result revealed that there was a gradual increase in bacterial count in all the treatments. There was however stimulation of growth of the organism in soil treated with 1% crude oil (Table 2). On the other hand, the treatment had little or no effect on the fungi count. There was however an initial depression of growth followed by a gradual increase in the fungi count of the 3 and 6% treated soil up to the last day of sampling (Table 3).

Percentage dry matter

The dry matter of *T. occidentalis* in soil treated with different concentrations of crude oil is shown in Figures 1 - 3. The percentage dry matter of the leaf is shown in Figure 1. There was no difference between the dry matter content of the control and 1% crude oil treated soil. However with the 3 and 6% treated soil, there was a significant reduction of the leaf dry matter. It was also observed that there was a luzurous leaf growth with the control and the 1% treatment compared to the growth in the 3 and 6% treated soils. Specifically the plants in the 6% treated soil which emerged late, developed few leaves which withered away before the 28^{th} day of planting as shown in Plates 1 - 3.

The percentage dry matter of the stem of *T. occidentalis* was equally affected by the crude oil treatment Figure 2. There was a significant reduction in the stem

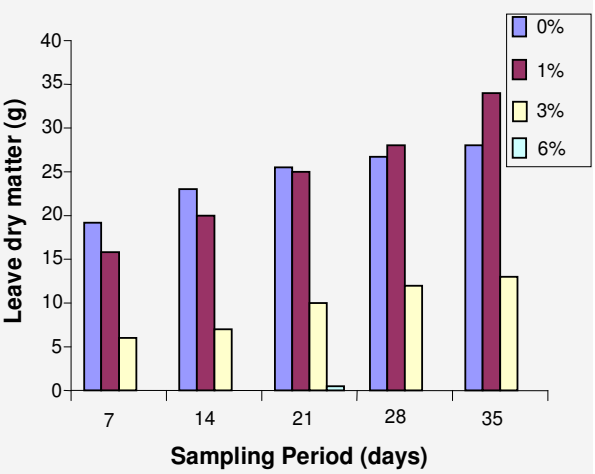

Figure 1. The percentage dry matter of leaves of *T. occidentalis* soil treated with different concentration of crude

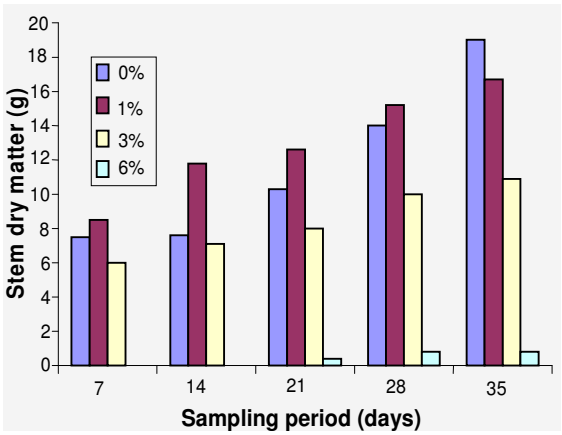

Figure 2. The percentage dry matter of stem of *T. occidentalis* in soil treated with different concentration of crude oil.

dry matter for the 3% and the 6% treatment compared to the control and the 1% crude oil treatment.

The percentage dry matter of root is presented in Figure 3. There was a gradual increase in the root dry matter for the 0% and 1% treated soils. This was however, not applicable to the 3% to 6% treated soils. There was a significant (P>0.05) reduction in the dry matter production of the root.

DISCUSSION

The effect of crude oil on microorganisms and the dry matter of fluted pumpkin (*T. occidentalis*) was carried out. Before the work, the soil was analysed to acertain the critical values of the essential nutrients. The analyses showed that the soil particle size was 93.8 - 9% for sand,

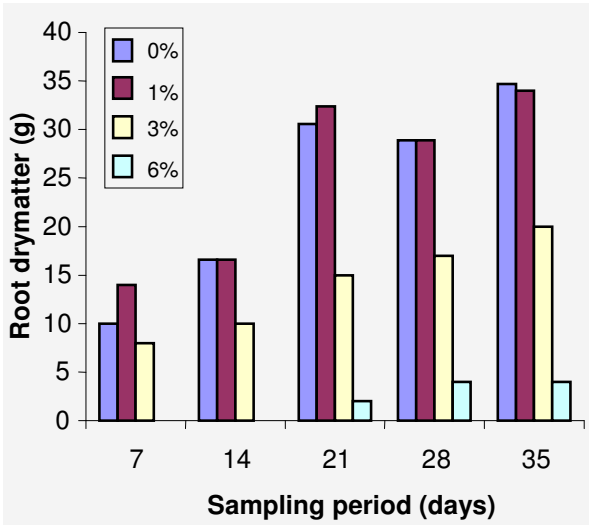

Figure 3. The percentage dry matter of root of *T. occidentalis* in soil treated with different concentration of crude oil.

1 - 2% for silt and 3.2 - 4.2% for clay. The essential soil nutrient, NPK had values of 1.72%, 44.66 mg/kg and 0.10%, respectively. The organic matter level was 3.0% with the pH of 6.40. The effective cation exchange capacity (ECEC) of the soil was 5.20 mol/kg. The analyses revealed that the soil used was a sandy soil and is known to belong to the Ultisol series of the USDA soil taxonomic system of classification (Ibia et al., 2002).

The study revealed that the higher crude oil pollution levels, 3 and 6% siginificantly delayed emergence and inhibit the germination of some seeds. The poor emergence obtained at higher pollution levels of the oil treatment was attributed to poor aeration. In addition, the inhibition of germination especially at the 6% pollution level may be due to the absorption of the oil by the seeds, which caused them to be swollen and slimmy, as was observed when the ungerminated seeds were escavated from the treated soil. This observation agrees with the work of Amakin and Onofeghara (1978) who working on the effect of crude oil on *Zea mays* and *Capsicum fruitescens* noted a significant decrease in the rate of germination. They attributed the inhibition primarily to the physical surface characteristics of the oil, which reduced contact of the seeds with water and oxygen. Similarly, Proffitt et al. (1995) also reported that seedlings of *Rhizophora mangle* and *Avicenia germinans* (Mangle) could only survive up to two weeks when treated with crude oil. Ekpo and Nwankpa (2005) also reported that while 1% soil pollution with crude oil enhanced the sprouting of ginger *Zingiber officinale*, 15% crude oil pollution completely suppressed the sprouting of ginger.

The result of the microbial count revealed that there was a gradual increase in both bacteria and fungi counts in all the treatments. There was a slight reduction in the number of the organisms in the 3 and 6% crude oil

Plate 1. Growth performance of fluted pumpkin on different concentration of crude oil (21 days post germination).

Plate 2. Growth performance of fluted pumpkin on different concentration of crude oil (28 days post germination).

Plate 3. Growth performance of fluted pumpkin on different concentration of crude oil (35 days post germination).

treatment. This effect was however shortlived, as both the bacteria and the fungi counts at the end of the experiment was significantly (P>0.05) higher in the higher oil treatments than in the control and the 1% treated soil. The initial suppression of the microbial count is attributed to the selective destruction of the microorganisms by the crude oil. This is because the crude oil produces an anaerobic condition as it is introduced into the soil and this automatically eliminates most of the aerobic organisms. This agrees with the report of Odu (1972) that crude oil introduced into the soil, causes initial damage to the soil biota.

The significant increase observed in this study stems from the fact that as most biodegraders recovered from the initial shock, they produced enzymes that were able to degrade the crude oil. This agrees with the report of Ekpo and Ekpo (2006) who working on the biodegradation of Bonny light and Bonny medium crude oil, noted that the initial outcome of a natural microbial population in contact with petroleum hydrocarbon is most often a reduction in the microbial biomas followed by an increase in biodegraders. Nakamura et al. (2007) reported the isolation of two microbial genera *Caulobacter* sp. and *Alcanivorax* sp. from the Russian oil spill that exhibited a high capacity of degrading both saturated and aromatic fraction of petroleum.

Similarly, Hozumi et al. (2000) reported the isolation of organisms with high potential for degrading oil with high viscosity after an oil spill. The increase therefore of the organisms in the higher pollution levels in this study is attributed to the utilization of the crude oil by indigenous hydrocarbon degraders as nutrient for their growth.

This study also revealed that the dry matter of the leaf, the stem and the root at higher levels of pollution were significantly (P>0.05) reduced compared to the control and the 1% crude oil treatment. There was a continous reduction in leaf number with increase in concentration of oil pollution. These reductions at higher levels of pollution may be attributed to interference of oil in the soil physical and chemical properties and subsequently the transpiration and photosynthesis in the plant.

The reduction in the leaf number of the crop as the pollution levels increases, can be related to the reduction in the total primary production of the crop, thus bringing about the reduction in the total dry mattr of the plant (Baker, 1971; Ekpo and Thomas, 2007).

It was observed from this study that the 1% crude oil treatment had better growth than the control (the

unpolluted soil). This increase is attributed to the fact that at lower level of pollution, crude oil acts rather as a source of nutrient to the organisms which when degraded enhance the fertility of the soil. This result agrees with the report of Odu (1981) who stated that crude oil pollution up to 1% could easily be degraded by natural rehabilitation and this will increase the organic matter in the soil, improve soil fertility with the physical and chemical properties of the soil.

It was specially observed that the 6% treatment had the worst impact on the dry matter. It did not only hinder germination until the second week of planting, there was a dramatic reduction in leaf number and subsequently leaf dry matter. The shoot also withered at the forth week after planting. This is attributed to the poor soil condition caused by the crude oil. This result agrees with the report of Anoliefo and Vwioko (1995) who noted that 84 days after planting, the mean height and leaf area of *Capsicum annum* in soil treated with 3% spent lubricating oil gave the lowest values. They also observed that *Lycopersicon esculentum* (Miller) in all the higher treatment with the soil had premature death.

Conclusion

In considering the above result, it is reasonable to state that crude oil at lower concentrations (1% and below) are beneficial to both microorganisms and plants, specifically the test crop *T. occidentalis*. Above this concentration, it was noted that the effect became variable. While at higher concentration it leads to the initial suppression of growth of microorganisms and subsequently acts as an enhancement to their growth, the reverse was the case with plants. At 3% it was fount to delay the germination of seed and a reduction in the dry matter of leaf, stem and root, while at higher concentration (6%), it lead to inhibition of germination or very poor germination and growth which was terminated prematurely. It should therefore be the concern of all and sundry to guard against crude oil pollution of our aerable agricultural land in other to maintain our quest for sustainable agricultural productivity.

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