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Impact of Crude Oil Polluted Soil on Seedling Morphological Characteristics and Biomass Accumulation of *Monodora myristica* (African Nutmeg)

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ABSTRACT: This study was designed to investigate the effect of crude oil polluted soil on the morphological characteristics and biomass accumulation of *Monodora myristica* seedling. The study was carried out in the forest nursery of the Department of Forestry and Wildlife Management, University of Port Harcourt, Nigeria using standard field experimental methods. Significant variations ($P \leq 0.05$) were observed in seedling height and biomass while number of leaves and collar diameter displayed no significant differences ($P > 0.05$) between treatments. The result shows that *M. myristica* seedlings are not tolerant to crude oil pollution. The trend in growth parameters measured showed that increased level of crude oil reduced growth. It is recommended that *M. myristica* seedlings should not be raised in crude oil polluted soils or be used in crude oil pollution remediation programmes due to its poor growth performance.

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Monodora myristica, commonly called African nutmeg or calabash nutmeg is a perennial edible plant of the family Annonaceae (Onyenibe *et al.*, 2015). It is primarily found in the evergreen forests of West Africa in nations like Liberia, Nigeria, Cameroon, Angola, Uganda and west Kenya (Onyenibe *et al.*, 2015) and its common names in Nigeria are ehuru, ariwo, ehiri and airama (Burabai *et al.*, 2008). Its seed is a common spice in West African cooking and has a taste and aroma comparable to nutmeg. This tropical shrub is of the family of flowering plants (Okafor, 1976). The fruit of *M. myristica* is a 20 cm diameter berry that is smooth, green, spherical, and when it ages, it turns woody. It is joined to an elongated stalk that can reach a length of 60 cm. The many long, pale brown seeds, which are about 1.5 cm long and encircled by a white, aromatic pulp, are found inside the fruit. Studies have shown that almost every part of *M. myristica* tree is important economically. The timber is hard, easy to work with and is used for carpentry, house fittings and joinery while the seeds are also made into necklaces (Nguefack *et al.*, 2004). Reports abound in the literature as to the medicinal use of *M. myristica*, the stem bark is used in the treatments of haemorrhoids, stomach ache, fever pains and eye diseases (Nwaoguikpe and Uwakwe, 2005), while the seeds are used in treating headache and hypertension in Central African Republic (Koudou *et al.*, 2007). In Eastern Nigeria, the seeds are used as condiment and is one of the major spices used as postpartum tonic. *M. myristica* has been proven to have anti-sickling properties (Nwaoguikpe and Uwakwe, 2005). When grounded to powder, the kernel is used to prepare soup as stimulant to relieve constipation and control passive uterine haemorrhage in women immediately after child birth (Udeala, 1980; Iwu *et al.*, 1987). Crude oil

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is a natural occurring complex mixture of hydrocarbon and non-hydrocarbon compounds and at high concentrations can be toxic to living organisms (Nelson-Smith, 1973). The discovery of crude oil in Nigeria has significantly improved her economy due to its importance as a significant source of foreign exchange and in boosting the Gross Domestic Product (GDP) of the country. However, its exploration and exploitation has posed immense danger and harm to the oil-producing communities due to the resultant effect it has on the aquatic life and soil topography of the oil-rich region. The Niger Delta region of Nigeria is well-known for the production of oil that commenced as far back as 1956 and the discovery of oil in the Niger Delta region improved the financial state of the economy but was immediately accompanied by a series of crude oil spillage on land and water giving rise to soil and water pollution (Adekunle and Adeniyi, 2015). The major cause of oil spills has been identified to be from vandalization and leakages of pipelines. The effects of environmental pollution resulting from the production of oil became a topical issue at both national and international levels (Adekunle and Adeniyi, 2015). Crude oil contamination in and around the exploration and spillage sites of oil industries is a burning problem throughout the world. Crude oil releases toxic hydrocarbons in nature which causes pollution. Soil contamination has become a global phenomenon. Chemicals and heavy metals have contaminated soil in many regions of the world as a result of industrial, agricultural, and uncontrolled waste disposal practices. (Tang and Angela, 2019). Contamination of soil with oil results in serious retardation of growth of most plants, primarily due to its effects on the physical and chemical properties of soil and soil water relations (Saini and Arya, 2016). There are several reports of the effect of crude oil pollution on plant growth (Osuji and Nwoye, 2007). Oil pollution causes loss of plant covering and plants diversity (Maranho *et al.,* 2006). As important as *M. myristica* is, its natural population is depleting very fast in the wild as a result of overexploitation, deforestation and forest degradation consequent upon many underlying causes many of which are no more obscure. Excessive pressure on tropical forests by people and their animals have posed a very serious challenge to natural regeneration of many tropical tree species because the mother trees that should serve as seed trees are being removed for one reason or the other (Longman, 1993). Ngwuta *et al*. (2016) reported that though *Monodora myristica* is one of the valued forest tree species, it is facing the threat of extinction caused by large scale exploitation and destruction of the natural forests. The silvicultural requirements of most forest tree species including *M. myristica* are inconsistent and results vary from location to location. Information on the silvicultural requirements of *M. myristica* have not been well documented. There is also no adequate information on the effect of crude oil pollution on *M. myristica* seedlings thereby creating need for this study.

There are compelling reasons why the research on the effect of crude oil pollution on seedlings morphological characteristics and biomass accumulation of *Monodora myristica* is necessary. Obtaining adequate knowledge on the impact of crude oil polluted soil on the seedlings of *Monodora myristica* is very essential to determining the level of pollution this species can tolerate. Findings from the study can inform the development of regulations and policies related to the protection of indigenous plants and ecosystems in regions where *Monodora myristica* is found. It can also contribute to guidelines for responsible resource extraction and environmental protection. Hence, the objective of this paper was to investigate the effect of crude oil polluted soil on the morphological characteristics and biomass accumulation of *Monodora myristica* seedling.

MATERIALS AND METHOD

Study Area: The study was carried out at the forest nursery of the Department of Forestry and Wildlife Management., Faculty of Agriculture, University of Port Harcourt, Rivers State, Nigeria. The nursery lies at Latitude 04°53˃38.3’N and Longitude 00.6° 54’38’E between October and April 2021.

Materials: Materials used for the study include: Viable seeds of *Monodora myristica*; germination basket and medium sized polybags for transplanting; a field notebook and pen for data collection; sterilized forest topsoil used as growth medium for transplanting seedlings; sharp sand used as medium for seed germination; and crude oil for polluting the soil.

Fruit collection and processing: The seeds of *Monodora myristica* were gotten from Okomu National Park in Edo State, Nigeria. The floatation method of seed viability test was employed to detect the viable seeds. Seeds that floated after putting them in a container filled with water were regarded as non-viable and discarded while the seeds that sank were regarded as viable and used for the experiment.

Experimental Design: The experiment was laid out in a completely randomized design (CRD). One hundred and fifty (150) seeds were directly sown in a germination tray filled with sharp sand. Watering was done as necessary. After one month of establishment, 10 seedlings were pricked into poly-bags filled with topsoil for each the nine treatments. The treatments involved polluting 10 kg of topsoil with different levels of crude oil (0 control), 25, 50, 75, 100, 125, 150, 175 and 200 ml). The topsoil was mixed thoroughly with different volumes of crude oil and left for 2 days to allow for proper mixing. Prior to the transplanting operation, the poly-bags were fully watered. Watering and weeding were carried out regularly and when required throughout the period of the experiment.

Seedling Growth Data Collection: Seedling growth data were collected on seedling height (cm), diameter (mm) and leaf number. The data collection began one month after planting and monthly thereafter for six months. The seedling height was measured from the substrate level to the tip of the youngest leaf using a meter rule; stem collar diameter was measured at the collar position using a digital vernier caliper; the numbers of fully expanded leaves were determined `by directly counting.

Seedling Biomass and Moisture Content: Five seedlings were chosen from each treatment at the end of the experiment and carefully removed from the polybags and the root system exposed by carefully washing off the growth media from the roots. Absorbent paper was used for blotting excess moisture from the plants. Seedlings were then separated into shoot and root components by cutting at the collar. The root length, fresh weight of shoot (including the leaves) and root were taken and then placed in a paper bag for drying. The shoot and the root samples were dried in the oven at 70°C for three days (72 hours). The biomass (g) was determined by weighing both wet and dry weight with a weighing scale while the moisture content was determined by subtracting the dry weight from the wet weight.

Data Analysis: Data collected were analyzed using SPSS statistical software (SPSS version 18, SPSS Inc.). One-way analysis of variance was used to test for significant differences ($p \leq 0.05$) in the evaluated seedling attributes among the treatments. Duncan multiple range test (DMRT) was used to determine the variation in means. All graphs were plotted using Microsoft excel 2010.

RESULTS AND DISCUSSION

Growth Response of M. myristica Seedlings to Crude Oil Pollution: Seedling Height: Seedlings of *M. myristica* showed considerable variations ($p \leq 0.05$) in height at all stages of growth. *M. myristica* seedlings under different levels of crude oil pollution showed a decrease in height as oil pollution increased with the Control having highest height from months 1 to 6 (21.74, 25.00, 25.77, 26.06, 26.43 and 30.58 cm, respectively), followed by 25ml (21.29, 24.10, 24.81, 25.18, 25.67 and 27.95 cm, respectively) while lowest height was observed at 200 ml (15.44, 17.69, 18.52, 18.98, 19.67 and 21.35cm) at all stages of growth (Table 1).

Table 1. Height performance of *M. myristica* seedling at different levels of crude oil pollution (μ±SE).

Levels of Crude oil pollution (ml)	Seedling Height (cm) (Months) 1	2	3	4	5	6
0	21.74±1.16a	25.00±1.71sa	25.77±1.90a	26.02±1.93a	26.43±1.87a	30.58±1.53a
25	21.49±1.18a	24.10±1.82ab	24.81±1.80ab	25.18±1.68ab	25.67±1.74ab	27.95±1.86ab
50	19.71±0.92ab	22.94±1.42abc	23.78±1.46 ab	23.95±1.48ab	24.30±1.13ab	27.78±1.41ab
75	18.45±1.50ab	22.09±1.36 abc	23.21±1.68 abc	23.38±1.70 abc	24.00±1.65 abc	26.29±0.88bc
100	18.34±1.82ab	21.04±2.21 abc	22.53±1.43 abc	23.36±1.11 abc	24.05±1.48 abc	26.21±1.23bc
125	18.09±1.73ab	20.63±0.97 abc	21.33±1.08 abc	22.94±1.31 abc	23.13±1.32 abc	25.80±0.81bc
150	17.86±1.89ab	20.19±1.70 abc	21.24±1.65 abc	21.29±1.03 abc	21.80±0.91bc	23.07±1.30cd
175	15.96±1.57b	19.00±1.86bc	19.93±1.76bc	20.79±1.33bc	21.721.13bc	22.750±0.83cd
200	15.44±1.30b	17.69±1.81c	18.52±1.62c	18.98±1.52c	19.67±1.32c	21.35±1.64d
Mean	18.56±0.52	21.41±0.59	22.35±0.56	22.77±0.52	23.43±0.50	25.75±0.51
P	0.044	0.050	0.047	0.037	0.037	<0.001

Means with the same alphabet on the same column do not differ significantly (p > 0.05).

Collar Diameter: M. myristica seedlings under different levels of crude oil pollution showed a decrease in collar diameter as oil pollution increased with the Control (0 ml) having the highest collar diameter from months 1 to 6 (2.38, 2.63, 2.73, 2.86, 3.02 and 3.31 mm, respectively), followed by seedlings grown in 25 ml level of crude oil. The lowest collar diameter was observed in seedlings grown in 200 ml (2.09, 2.30, 2.41, 2.55, 2.64 and 2.84 respectively). However, the variations in collar diameter were not significant ($p > 0.05$) at all stages of growth (Table 2).

Leaf Production: Seedlings of *M. myristica* showed no significant variations ($p \leq 0.05$) in leaf number at all stages of growth. At month 6, the highest leaf production was observed in the Control (0 ml) while the lowest was observed in 200 ml level of crude oil. At month 2, the highest leaf production was observed in the Control (0ml) followed by 25ml but the lowest was observed in 200ml. This is shown in Figure 1.

Table 2. Collar diameter of *M. myristica* seedling at different levels of crude oil pollution (μ±SE).

Levels of Crude oil pollution (ml)	Seedling Collar Diameter (mm) (Months) 1	2	3	4	5	6
0	2.38±0.10	2.63±0.11	2.73±0.15	2.86±0.15	3.02±0.12	3.31±.1449
25	2.37±0.13	2.60±0.17	2.72±0.11	2.85±0.14	3.02±0.10	3.29±.1709
50	2.31±0.14	2.56±0.14	2.72±0.13	2.79±0.14	2.93±0.18	3.24±.1839
75	2.24±0.10	2.49±0.11	2.67±0.13	2.78±0.11	2.89±0.11	3.22±.1315
100	2.24±0.10	2.44±0.12	2.59±0.13	2.69±0.13	2.87±0.12	3.14±.1301
125	2.22±0.10	2.42±0.12	2.57±0.15	2.67±0.15	2.84±0.13	3.13±.0989
150	2.17±0.10	2.40±0.08	2.52±0.10	2.66±0.11	2.80±0.12	3.00±.1640
175	2.11±0.04	2.40±0.12	2.46±0.09	2.61±0.07	2.71±0.09	2.95±.1400
200	2.09±0.12	2.30±0.08	2.41±0.13	2.55±0.13	2.64±0.15	2.84±.1771
Mean	2.24±0.03	2.47±0.04	2.60±0.04	2.72±0.04	2.86±0.04	3.12±.0508
P	0.436	0.600	0.501	0.675	0.427	0.337

Means with the same alphabet on the same column do not differ significantly (p > 0.05).

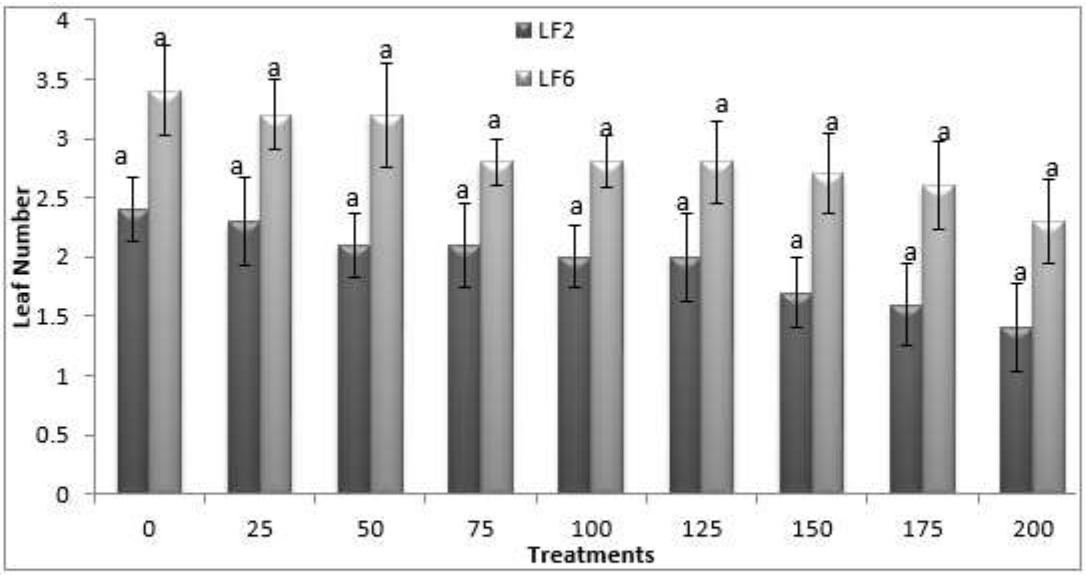

Fig 1: Effects of crude oil pollution on leaf production of *M. myristica* at months 2 and 6. Bars with the same letter (s) are not significantly different at the 0.05 level.

Table 3 Biomass of *M. myristica* seedlings at different temperature (μ±SE).

Levels of Crude oil pollution (ml)	Plant Biomass (g) SFW	SDW	SMC	RFW	RDW	RMC
0	2.06±0.37a	0.66±0.12a	1.40±0.26a	0.94±0.11a	0.30±0.02a	0.64±0.10a
25	1.79±0.17ab	0.57±0.03ab	1.22±0.21ab	0.91±0.12ab	0.28±0.04ab	0.64±0.14a
50	1.79±0.11ab	0.54±0.05abc	1.25±0.15ab	0.89±0.05ab	0.27±0.01ab	0.62±0.05ab
75	1.78±0.21ab	0.43±0.05bcd	1.35±0.18a	0.85±0.04ab	0.26±0.05abc	0.59±0.05ab
100	1.48±0.19abc	0.42±0.11bcd	1.07±0.15ab	0.81±0.05ab	0.23±0.02abc	0.58±0.04ab
125	1.39±0.19bc	0.41±0.06bcd	0.98±0.13ab	0.72±0.08abc	0.20±0.02bcd	0.52±0.10ab
150	1.09±0.16cd	0.35±0.06cd	0.75±0.10bc	0.65±0.06bc	0.20±0.04bcd	0.46±0.10ab
175	0.71±0.18de	0.25±0.06de	0.46±0.12cd	0.55±0.10c	0.18±0.03cd	0.42±0.02ab
200	0.32±0.03e	0.12±0.02e	0.20±0.01d	0.53±0.02c	0.13±0.01d	0.35±0.07b
Mean	1.38±0.10	0.42±0.03	0.9631±0.08	0.76±0.03	0.23±0.01	0.54±0.03
P	0.000	0.000	0.000	0.002	0.002	0.157

Means with the same alphabet on the same column do not differ significantly (p ≤ 0.05).
SFW = Shoot Fresh Weight; SDW = Shoot Dry Weight; SMC = Shoot Moisture Content; RFW = Root Fresh Weight; RDW = Root Dry Weight; RMC = Root Moisture Content

Plant Biomass: Seedling biomass of the different crude oil levels is presented in Table 3. There were significant differences ($p \leq 0.05$) in shoot fresh and dry weights, root fresh and dry weights and shoot moisture content but there was no significant difference ($p > 0.05$) in root moisture content among treatments. Plants biomass (fresh weight, dry weight and moisture) were highest in control and lowest in 200 ml.

Toxicity symptoms observed in plants exposed to oil pollution include stunted growth, suppression of leaves, chlorosis, necrosis, enormous reduction in biomass to stomata abnormalities (Baker, 1970). Effect of crude oil levels on seedling height and biomass were observed to be significantly different while collar diameter, number of leaves and root moisture content were not significantly different although it was observed that increase in crude oil levels led to decrease in growth for all parameters measured which shows that crude oil had adverse effect on plants. This may be attributed to disruption in water and nutrient uptake owing to the effects of oil in soil, and the depletion of soil nitrogen and phosphorus content (Baran *et al*., 2002). The reduction in the evaluated plant growth parameters with the increased crude oil levels could be related to increased effect of the crude oil which could have inhibited cell division, enlargement and expansion and nutrient availability. These could have negatively affected the anatomical and physiological structures of the plant and hence growth reduction (Agbogidi and Eshegbeyi, 2006). Growth reduction in the presence of crude oil has also been reported by other researchers including Bamidele *et al*. (2007), Chima *et al*. (2018) and Oyedeji *et al.* (2012). The trend in seedling height increment of *M. myristica* showed that the addition of various levels of crude oil retarded growth in total height. The observed reduction in the height of *M. myristica* agrees with the findings of Rowell (1977) that growth retardation is possible with oil pollution of soil due to insufficient aeration caused by displacement of air from pore spaces. This may have been caused by the higher quantity of oil present in the soil which may have inhibited the flow of nutrients. Growth reduction with increasing pollution level is suggestive of the fact that oil in the soil affects nutrient level probably as a result of immobilization (Agbogidi *et al*., 2009). Also, highest biomass observed in control which decreased as concentration increased agrees with the report of Baruah *et al.* (2016) that after harvest, plant biomass of *Crotalaria pallida* planted in soil contaminated with crude oil was found to be the maximum in 0 ppm (i.e. the Control) and gradually decreased according to their concentration gradient. The non-significant difference in collar diameter and leaf production among treatments suggests that crude oil-pollution of soil up to 200 ml/10kg of soil had no negative impact on the growth parameters for the six-month period of the experiment.

*Conclusion***:** Crude oil pollution significantly affected the early growth of *M. myristica* in height, collar diameter, leaf production and biomass. The result shows that *M. myristica* seedlings are not tolerant to crude oil pollution. It is recommended that *M. myristica* seedlings should not be raised in crude oil polluted soils or be used in crude oil pollution remediation programmes or projects due to its poor growth performance in crude oil-polluted soil.

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