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Phytotoxicity activities and chemical composition of the seed essential oil of Monodora myristica

Awojide H. Shola[1*], Ajayi T. Mercy[1], Oyewole A. Kehinde[2], Adeyemo G. Abayomi[1], Fadunmade E. Olumide[1], Adebayo E. Adeleke[3], and Abdullahi M. Tolani[1]

[1]*Department of Pure and Applied Chemistry, Faculty of Basic and Applied Sciences, Osun State University, Osogbo, Nigeria*

[2]*Department of Chemical Engineering, Osun State University, Nigeria*

[3]*Department of Basic Sciences, Adeleke University, Ede Osun State, Nigeria*

**Corresponding author (shola.awojide@uniosun.edu.ng)*

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ABSTRACT

The phytotoxic effect of essential oil from African nutmeg (Monodora myristica) seeds on growth of tomato (Solanum lycopersicum L)., maize (Zea mays), and cowpea (Vigna unguiculata) was examined. Essential oil from the seeds of M. myristica was extracted by hydrodistillation. The essential oil's composition was examined by gas chromatography-mass spectrometry (GC-MS). The phytotoxicity of the essential oil formulation at different concentrations for five days was evaluated on the leaves and roots. The activity was ascertained by estimating the shoots' and the roots' lenght. The result of GC-MS showed α–phellandrene (43.2%) as the essential oil's main constituent. The essential oil reduced the growth of the root and shoot of tomato, maize, and cowpea. At 4 mL/L treatment, the percentage inhibition of roots ranged from 63.8 to 75.4% after five days, while that of the shoot was 100% after three days. A varying toxicity was reported on the leaves of the seedlings of the plants after 24 hours. Phytotoxic was more potent on the leaves of cowpea and lowest in tomato. Phototoxic activity was also noted when essential oil was administered to the Seedling's roots. The three species showed notable phytotoxicity when exposed to the essential oil of M. myristica.

1. INTRODUCTION

Agriculture has been a major means of feeding the world. However, crop production faces both biotic and abiotic constraints. Abiotic factors such as salinity, temperature, and natural disasters affect net crop production, but these factors do not constantly contribute adversely to crop production. Biotic factors unlike the abiotic factors place daily restriction on farmers (Awojide et al., 2021). Agricultural production has been harmed by weeds (Gharde et al., 2018; Kumar & Dwivedi, 2018). In production systems for food, vegetables, medicinal, and ornamental crops, weeds are often a major problem (El-Wakeel et al., 2020). Weeds can compete for space, water, light, gases, and nutrients with crop plants. They act as secondary hosts for pests, outgrow crop plants and produce toxic substances that weaken the growth of the associated crops. This increases labor and other agricultural expenditures, which consequently leads to reductions in the quantity and quality of agricultural produce (Zimdahl 2018; El-Masry et al., 2019). Weeds have been the major threat to agriculture, as weed management practices significantly cost about 40 billion dollars annually (Girdhar et al., 2014a; Abouziena & Haggag, 2016; Prakash & Kumar, 2017).

For weed management, several practices have been adopted, some of which include: (a) preventive measures, (b) cultural practices (c) mechanical methods, (d) plant breeding, (e) biotechnological strategies: use of transgenic approaches, (f) biological control, (g) mulching, (h) soil solarization, and (i) chemical methods (Meena et al., 2017). However, the chemical method, which is the application of herbicides, has been one of the most liked means of weed control owing to its performance (Singh et al., 2018). Herbicides are largely used by both local small-scale and large-scale crop producers (Obiri et al., 2021), but their use results in detrimental effects on crops, humans, and animals (Jabran et al., 2015). Excessive herbicides uses results in the contamination of soil and water with noxious remnants which accumulate in farm produce, weeds also develop resistance to these herbicides (Böcker et al., 2019).

With much concern about the negative impact of chemical herbicides on plants and humans, there is an increasing demand for eco-friendly pesticides with ecosystem safety and specific mechanisms of action (El-Wakeel et al., 2019a). Natural products (NPs) produce a major chemical used in farming for a variety of chemical applications that bear little resemblance to chemical synthesis (Awojide et al., 2021). Various herbicides, such as drug products, are derived from NPs (Dayan & Duke, 2014). Numerous plants are found to produce secondary metabolites known as allochemicals, which have selective herbicidal properties (El-Masry et al., 2019; El-Rokiek et al., 2014; El-Wakeel et al., 2019a; El-Wakeel et al., 2019b). This harmful or beneficial effect is known as the allopathy phenomenon (Reigosa et al., 2006). At certain concentrations, some of these compounds are phytotoxic to some plants and stimulatory to others. The allopathic extracts could help to put a check on the growth of unwanted plants (El-Wakeel et al., 2019a and El-Wakeel et al., 2019b).

African nutmeg (*Monodora myristica*), commonly referred to as ‘Ariwo’ in South -Western Nigeria, is a member of the Annonaceae family and perennial food flora that is categorized as Annonaceae (Awojide et al., 2023). The extract from the seed contains not only useful pharmacologically active components such as polyphenols, alkaloids, flavonoids, and vitamins, as well as innumerable other compounds but also useful lipids (Anna & Muthulisa, 2017; Queency et al., 2022). African nutmeg possesses both medicinal and economic benefits (Onyenibe et al., 2015). Its aperient, stomachic, tonic, antiemetic, and stimulating effects and the high mineral and essential oil contents give sustaining and meaningful advantages (Akpojotor & Kagbo, 2016). The seeds are abundant in steroids, alkaloids, saponins, and some other secondary metabolites (Anna & Muthulisa, 2017). Africa an underdeveloped continent, is faced with the problem of combating weeds. The use of synthetic chemicals has been the focus of farmers; it will be of great advantage to work for eco-friendly ways of controlling this menace to help the local farmers. A literature report on the herbicidal activeness of essential oil from African nutmeg is uncommon in Africa. Though the plants used for this pilot research are commercial crops, the selection was based on the variation in growth morphology (monocotyledon and dicotyledon). The result of the study will make room for more research on weeds. Our study focused on assessing the phototoxicity of *Monodora myristica* oil against *Solanum. lycopersicum L.* (tomato), *Zea mays* (maize), and *Vigna unguiculata* ((cowpea).

2. MATERIALS AND METHOD

2.1. Materials

African nutmeg seeds were bought at Oja-Oba market in Oshogbo, Osun State, Nigeria, 7.7654°N, 4.554°E. The seeds were identified with an herbarium specimen whose voucher number is IFE-17945, at the Botany Department of Obafemi Awolowo University. The African nutmeg seed was decorticated, aero-dried and pulverized to powder. The seeds of cowpea, maize and tomato were all acquired in Ibadan, Oyo State, Nigeria, at the International Institute of Tropical Agriculture (IITA). At IITA, the seeds were identified by comparison with specimens whose voucher numbers were IT99K, IT123K, and IT120K, respectively.

2.2. Methods

2.2.1. Essential oil extraction

The milled seeds of African nutmeg were subjected to hydro-distillation Drying of the essential oil was done with with the aid of $NaSO_4$(anhydrous). The essential oils obtained were preserved within sealed containers and kept at a temperature of 4°C (Awojide et al., 2023).

2.2.2. GC-MS Analysis

Agilent 6890N instrument coupled with a flame ionization detector and capillary column HP-5MS (30.0m × 0.250mm × 0.250µm). The constituents were recognized on an Agilent Technologies 5973N mass spectrometer. The GC was set to a temperature of 60°C for 15 minutes, increased to 280°C at 5°C/min, and then 280°C for 15 min. The injector was set at a temperature of 270°C while essential oil of 1 µL was injected with helium as the carrier gas. A flow rate of 1.0 mL per 60 seconds was maintained. The range of Spectra scanned was from 20-550 m/z at 2 scans s^{-1}. The individual components analyzed by MS and their constituents' identity were compared with their Kovat's retention index in relation to hydrocarbons relative to C8–C32 n-alkanes and mass spectra using reference samples or with information already included in the NIST 2008 mass spectra, which are accessible through the library and published works.*2.2.3. Planting of seedlings*

Sand and loamy soil were poured into plastic cups up to two centimeters from the edge. The soil in the plastic cup was filled with around 50 milliliters of water. Each cup contained five cowpea seeds, spaced two centimeters apart in a circular pattern. Thereafter, a generous pinch of earth was applied over the seeds. The seedlings that were sown were kept in a greenhouse. (temperatures between 18°C and 24°C as well as relative humidity of 50%) to allow the seedlings to grow sufficient foliage (Shiv et al., 2003). This procedure was used for the planting of tomato and maize.

2.3. Herbicidal Activity

2.3.1. Herbicide Formulation

Concentrations of 1, 2, 3, and 4 mL/L were achieved by dissolving 5, 10, 15, and 20 µL of the essential oil in 4 mL of deionized water and 1 mL of acetone as an emulsifier, a liquid herbicide mixture containing the essential oil was produced. One milliliter of acetone was mixed in four milliliters of deionized water to create a control formulation.

2.4. Growth Inhibitory Test

About ten seeds of each plant species (tomato, maize, and cowpea) were used for each formulation. To facilitate faster germination, the seeds were first immersed in water for a period of two hours, which helped to soften their outer layerThe seeds were then placed on cotton wool in a petri dish and the concentrations were applied to each seed in the petri dish. The experiment was replicated three times for each treatment and the growth measurement of the root and shoot was performed daily for five days.

The percentage inhibition of the root and shoot was calculated with the equation below:

Percentage of Inhibition rate $= \frac{C-T}{C}$ x100

where C is the length of the control seedlings' shoots or shoots and T is the length of the treated seedlings' shoots or roots.

2.5. Soil Application

Five treatments (0, 1, 2, 3 and 4 mL/L) were applied to ten seeded plants of each of the plant species and replicated three times. At the greenhouse, the formulation was sprayed on the soil close to the roots of the seedlings, this was done in order to facilitate the herbicide's formulation reaching the root. The quantity of distressed plants that were visible in the pot after 24 hours was recorded.

2.6. Foliar Application

Five treatments (0, 1, 2, 3 and 4 mL/L) were applied to ten seeded plants of each of the plant species and replicated three times. To enable the herbicide formulation to reach the leaf the seedlings were sprayed with the formulation., and the number of plants in the pot showing signs of distress after 24 hours was recorded. The leaf surface's capacity to hold the solution determined how much solvent was utilized.

2.7. Statistical analysis

The research was conducted in triplicate, and SPSS package was utilized for data analysis, specifically analysis of variance (ANOVA). When a difference was P≤0.05, it was deemed significant.

3. RESULTS AND DISCUSSION

3.1. Results

A yield of 0.62% was obtained from the extraction of the seeds of African nutmeg. Table 7 shows the prominent chemical compounds observed in the essential oil of African nutmeg. The composition percentage of α –phellandrene, O-cymene, Sabinol-cis, linanool and ethyl hexadecanoate were; 43, 2, 12.5, 12.3, 8.21 and 6.70 respectively.

In a dose and time-response manner, the essential oil reduced the root growth of maize (Table 1). The initial inhibitory activity was recorded after two days, showing an increase in inhibitory effects with increasing concentration of essential oil. At a maximum concentration of 4 ml/L, 48.0% of inhibitory activity on the maize root was observed after two days. The maximum t root inhibition of 63.8% was observed after five days, Although the control trial showed no inhibitory after five days of experiment. The root inhibitory activity of the oil formulations intensified with increasing concentration.

The essential oil's inhibiting action against cowpea root is shown in Table 2. The outcome in the table demonstrated that the earliest indication of inhibitory action was observed after the first day of treatment, and the concentration dosage recorded varied levels of activity. The inhibitory activity of cowpea root at 1mL/L concentration was 1.3%, while 52.3% was observed for 4 mL/L concentration after one day of treatment, which gave a dosage-dependent relationship. The highest level of inhibitory activity was reported at 4mL/L concentration on the fifth day of the experiment, with a percentage of inhibition of 75.4%. However, all concentrations reduced the rate of germination except the control.

Table 3 revealed the essential oil’s inhibitory action against tomato root. The result showed that the inhibition was time and dosage-dependent. The initial inhibition of 20.2% was recorded after day 1 with a 2 mL/L formulation. The highest inhibition of 78.3% was observed at 4mL/L concentration after five days of treatment.

The inhibitory activity of the oil on the shoot growth of cowpea, maize, and tomato is shown in Tables 4, 5 and 6, respectively. On the first three days of the experiment, there was no record of inhibitory activity of the root with all graded concentration of essential oil from African nutmeg. However, on the fourth day, inhibition of the root was recorded with 1mL/L concentration on maize, cowpea and tomato with (30.2%, 33.6%, and 33.4%), respectively. With 4mL/L concentration, 100% inhibitory activity was recorded for cowpea and tomato shoots respectively except for the maize shoot, which recorded maximum inhibition only after the 5th day.

Throughout the period of the experiment, the roots and shoots of maize, cowpea and tomato grew normally with the control formulation, showing no sign of phytotoxicity.

Figure 1 gives a graphical representation of the effect of essential oil on the leaves of maize, cowpea, and tomato. The figure shows that with the concentration of 2 mL/L, phototoxic effect on the leaves of the seedlings was clear by the discolouration of the leaves, showing a 20%, 60%, and 100% foliar effect on seedlings of tomato, maize and cowpea respectively, after 24 hours. The phototoxic effect was clear with all tested concentrations on leaves of maize, cowpea and tomato. Tomato was observed to have the least phototoxic effect and recorded a 40% effect on the leaves with 4 mL/L formulation.

Figure 2 illustrates a graphical representation of the root zone inhibitory activity, percentage of plants showing signs of distress after application of the formulation to the root base was observed. The results showed that at the lowest concentration of 1mL/L, tomato, maize and cowpea recorded 20%, 40%, and 100% root zone toxicity, respectively, after 24 hours. 100% root zone toxicity was recorded at all tested concentrations against cowpea after 24 hours. Tomato recorded the least root zone inhibition of 70% with a 4 mL/L concentration.

3.2. Discussion

The prominent components in the seed of African nutmeg were shown to contain *α*-phellandrene, limonene and pinene, while the essential oil from the leaves was shown to contain a high content of *β*-caryophyllene and humulene (Miediegha et al., 2022). According to Miediegha et al. (2022), the major components of the oil from the stem were γ-cadinene (31.31%) and α-elemene (17.98%), while the essential oil from the seed had germanene (25.48%) as one the major components. The differences in the chemical composition from those reported by other researchers could be because of environmental factors. These environmental factors could be because of the time of harvest, conditions the plants or seeds were subjected to after harvest, genetic factors in growth, and physiological variations (Cecilia et al., 2021). The phototoxicity of the essential oil could be attributed to the higher content of monoterpenes (Wojtunik-Kulesza, 2022).

Generally, essential oils are safe for the environment, animals, and people. They are useful in medicine, cosmetics, and food industries (Azirak and Sengul 2008). In the current research, the phytotoxic effect of essential oil from the African nutmeg was tested against tomato, maize and cowpea. The essential oil from *M. myristica* displayed inhibitory behavior on the growth of seeds (root and shoot) for tomato, maize and cowpea compared to the control experiment, which had no essential oil. This may be because of the presence of mono-terpenes in the oil (Silva et al., 2017). The oil retarded the growth of root and shoot at all tested concentrations. This report was confirmed by Awojide et al. (2021) who showed that the essential oils from three species of plants (*Cymbopo goncitratus, Micromeria fruticosa and Origanum syriacum*), had potential to inhibit wheat seed germination.

Among all, *Z. mays* had the lowest inhibition percentage for root and shoot growth, while tomato had the highest inhibition percentage for the root. The cowpea had the highest percentage inhibition for the experiment on the shoot. This result is similar to the report given by Awojide et al. (2021) in which essential oil from *Piper nigrium* had higher root inhibition against tomato than maize. Ibáñez and Blázquez (2018) further stated that when tested with peppermint essential oil, tomatoes displayed higher toxicity than corn and riceThe outcome showed that the phytotoxic effects of essential oil are more dominant on the roots than the shoots at all tested concentrations. Silva *et al.* (2017) also reported that both *Eucalyptus saligna* essential oil and extract reduce root growth more than shoot growth. Several studies have equally asserted that the roots of seedlings are more vulnerable to phytotoxins than the shoot (Bashar et al., 2023) Yoshimura et al. (2011) reported that the surface of plant shoots has a thick layer, which makes it less permeable to concentrations compared to the root.

The phytotoxic effect of oil was clear in the discoloration of leaves of tomato, maize and cowpea. Cowpea had the highest effect on leaves in all doses, while tomato had the least effect in all concentrations. Oxidative damage can cause loss of pigmentation, which could cause a serious reduction in the amount of chlorophyll content (Niu et al., 2013) and hence negatively influence photosynthesis.

Application of the oil on the root zones of tomato, maize and cowpea resulted in distress on the root zones of the plant seedlings. The number of seedlings that could not remain erect after the application of the concentration was recorded and converted into a percentage. Tomato had the least phototoxicity effect with all concentrations used, recording 70% maximum activity, while cowpea recorded the highest phototoxicity effect of 100% with all doses.

Table 1. Inhibition percentage of root growth of maize by the essential oil

Duration Time(Days)	Concentration (ml/L)				
	1	2	3	4	Control
1	$0.0±0.0^a$	$0.0±0.0^b$	$0.0±0.0^c$	$0.0±0.0^d$	$0.0±0.0^a$
2	$19.0±5.8^b$	$40.0±5.8^c$	$44.0±5.8^d$	$48.0±5.8^e$	$0.0±0.0^a$
3	$19.4±5.8^b$	$43.2±5.8^c$	$48.4±5.8^d$	$50.6±5.8^e$	$0.0±0.0^a$
4	$26.6±10.0^b$	$44.2±10.0^c$	$48.9±5.8^d$	$50.0±5.8^e$	$0.0±0.0^a$
5	$43.3±5.8^b$	$60.0±5.8^c$	$63.0±5.8^d$	$63.8±5.8^e$	$0.0±0.0^a$

Values inside a row with the same letter are not substantially different at $p<0.05$, as shown by the mean ± SD of three replicates

Table 2. Inhibition percentage of root growth of cowpea by the essential oil

Duration Time(days)	Concentration (ml/L)				
	1	2	3	4	Control
1	$1.3±0.02^a$	$18.3±5.8^b$	$38.7±5.8^c$	$52.3±3.2^d$	$0.0±0.0^a$
2	$25.4±5.8^b$	$40.6±5.8^c$	$50.8±5.8^d$	$60.5±4.1^e$	$0.0±0.0^a$
3	$39.2±5.8^b$	$53.6±5.8^c$	$57.1±5.8^d$	$64.3±5.8^e$	$0.0±0.0^a$
4	$51.1±6.2^b$	$62.3±5.8^c$	$65.4±5.8^d$	$71.4±5.8^e$	$0.0±0.0^a$
5	$58.3±5.8^b$	$67.8±5.8^c$	$70.2±5.8^d$	$75.4±5.8^e$	$0.0±0.0^a$

Values inside a row with the same letter are not substantially different at $p<0.05$, as shown by the mean ± SD of three replicates

Table 3. Inhibition percentage of root growth of tomato by the essential oil

Duration Time(days)	Concentration (ml/L)				
	1	2	3	4	Control
1	1.8±0.20[a]	20.2±5.8[b]	42.3±5.8[c]	55.1±5.8[d]	0.0±0.0[a]
2	27.3±5.8[b]	43.3±5.8[c]	53.4±5.8[d]	64.3±5..8[e]	0.0±0.0[a]
3	43.4±5.8[b]	57.4±5.8[c]	60.3±5.8[d]	68.1±5.8[e]	0.0±0.0[a]
4	57.3±5.8[b]	65.2±5.8[c]	69.1±5.8[d]	74.2±5.8[e]	0.0±0.0[a]
5	61.1±5.8[b]	68.3±5.8[c]	73.4±5.8[d]	78.3±5.8[e]	0.0±0.0[a]

Values inside a row with the same letter are not substantially different at p<0.05, as shown by the mean ± SD of three replicates

Table 4. Inhibition percentage of shoot growth of cowpea by the essential oil

Duration Time(days)	Concentration (ml/L)				
	1	2	3	4	Control
1	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
2	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
3	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
4	33.6±5.8[b]	70.6±10.0[c]	100±0.0[d]	100±0.0[d]	0.0±0.0[a]
5	50.7±10.0[b]	82.5±10.0[c]	100±0.0[d]	100±0.0[d]	0.0±0.0[a]

Values inside a row with the same letter are not substantially different at p<0.05, as shown by the mean

± SD of three replicates

Table 5. Inhibition percentage of shoot growth of maize*Z. mays* by the essential oil

Duration Time(days)	Concentration (ml/L)				
	1	2	3	4	Control
1	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
2	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
3	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
4	30.2±10.0[b]	40.6±5.8[c]	70.8±10.0[d]	100±0.0[e]	0.0±0.0[a]
5	50.7±10.0[b]	82.5±10.0[c]	80.3±5.8[d]	100±0.0[e]	0.0±0.0[a]

Values inside a row with the same letter are not substantially different at p<0.05, as shown by the mean ± SD of three replicates

Table 6. Inhibition percentage of shoot of tomato African nutmeg by the essential oil

Duration(days)	Concentration (ml/L)				
	1	2	3	4	Control
1	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
2	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
3	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]
4	33.4±5.8[b]	70.5±10.0[c]	100±0.0[d]	100±0.0[d]	0.0±0.0[a]
5	49.3±10.0[b]	81.5±10.0[c]	100±0.0[d]	100±0.0[d]	0.0±0.0[a]

Values inside a row with the same letter are not substantially different at p<0.05, as shown by the mean ± SD of three replicates

Table 7. Components observed from the GC/MS analysis of the essential oil of African nutmeg

Component	Percentage	Retention Time (Min)	RI [Calculated]	RI [Literature]
α –phellandrene	43.2	8.2	993.3	999.1
Sabinol-cis	12.3	5.8	1128.2	1135.6
Linanool	8.21	3.2	1089.6	1099.0
Ethyl hexadecanoate	6.70	17.5	1987.4	1991.5
Methyl oleate	5.32	18.3	2075.2	2081.3
Sabinol	3.08	5.5	1123.2	1139.1
o-Cymene	12.50	8.4	1010.3	1012
Carvacrol acetate	1.31	8.2	1350.2	1354.4
δ-Cadinene	1.66	10.1	1511.1	1513.9
γ-Cadinene	1.32	9.2	1498.1	1505.7
Cadinol	0.21	10.94	1634.1	1640.2
Caryophyllene oxide	0.09	10.9	1563.3	1570.0
Cis-beta-Terpineol	1.11	3.50	1120.3	1129.3
β-Bisabolol	0.52	11.10	1648.4	1658.6

RI (literature) represents the retention indices as reported by Babushok et al., 2011. Retention indices of components for dimethylsilicone stationary phase.

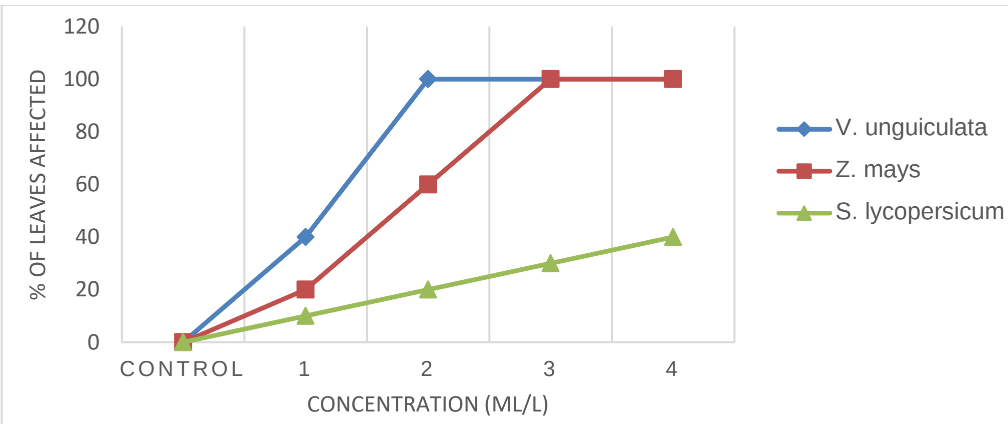

Figure 1. Percentage Folia Activity of African nutmeg essential oil on *cowpea, maize* and tomato after day one

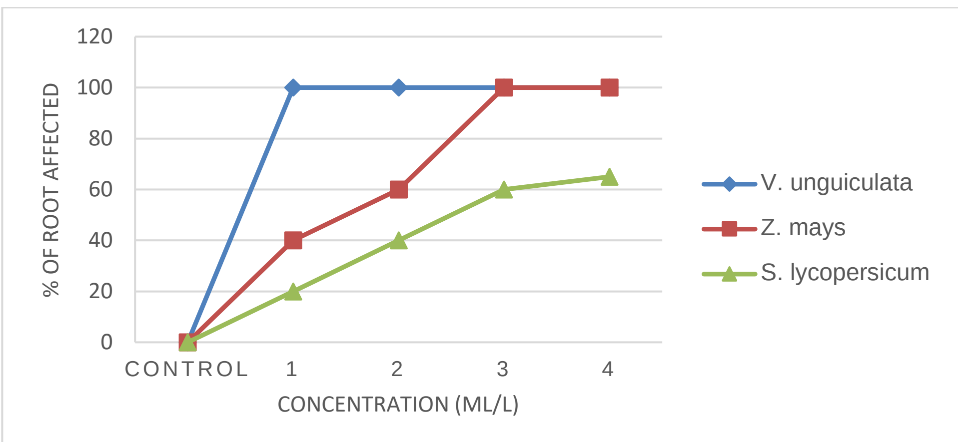

Figure 2. Percentage Root Zone Inhibitory Activity of African nutmeg essential oil on cowpea, *maize* and tomato after day one

4. CONCLUSION

In this study, essential oil from African nutmeg shows a strong phytotoxic effect on the leaves and root zone of the three plants. The essential oil also inhibited the growth of the root and the shoot of the seedlings of the three plants used in this study, but the essential oil's effects were based on both dose and timing. The phytotoxicity also varied with the plant species. The biodegradability of the essential oil could make it a source for developing bio-herbicides, which will help reduce the effects of the synthetic herbicides. The wide spectrum of activities on different plant species with different morphologies will make it useful for combating different weeds.

For sustainable weed management in Africa, and assuaging the challenges encountered by local farmers, it is necessary to conduct research on this plant-based extract, which could provide novel compounds as herbicides.

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