

Allelopathic Activities and Chemical Composition of Essential Oil from *Piper umbellatum* as a Potential Bio-Herbicide in Africa

S. H. Awojide*, E. O. Fadunmade, W. B. Agbaje, A. S. Tayo, I. S. Adedotun, G. A. Adeyemo and M. T. Abdullahi

Department of Pure and Applied Chemistry, Osun State University, Osogbo, Nigeria

*Corresponding Author: shola.awojide@uniosun.edu.ng

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ABSTRACT

The phytotoxicity of the essential oil of the seeds of *Piper umbellatum* against *Vigna unguiculata*, *Solanum lycopersicum* L and *Zea mays* was accessed. The essential oil was obtained by hydrodistillation while the constituent of the essential oil was determined by Gas Chromatography-Mass spectroscopy. Herbicidal activities were conducted to determine the growth inhibitory activity, foliar and root zone activities. The essential oil of *P. umbellatum* displayed some potentials for growth inhibition of the shoot and root of the three crops. The shoot was fully inhibited after 72 hours while inhibition of the root ranged from 60.4 - 63.2% after 144 hours using 4 ml/l concentration. The main constituents of the *P. umbellatum* essential oil were identified as Caryophyllene (10.44%), Aromadendrene (13.74%), γ -Bisabolene (8.06%), β -Sesquiphellandrene (4.58%), and Nerolidol (3.47%). Sesquiterpenes were found in the oil as abundant components. Toxicity on the leaves of the seedlings of *S. lycopersicum*, *Z. mays* and *V. unguiculata* were recorded at varying degree of phototoxic effects after 24 hours. Phytotoxicity activity on the leaves of the seedling was highest in *V. unguiculata* and lowest in *S. Lycopersicum* L. The phototoxic effect was visible when the essential oil was used on the root zone of the seedlings of *Vigna unguiculata* L., *Solanum lycopersicum* L and *Zea mays*. Maximum toxicity was observed for the seedlings of *Z. mays* and *V. unguiculata* L when 2 ml/l of the graded concentration was applied after 24 hours. Essential oil from *P. umbellatum* can be seen as a possible bio-herbicide with a wide range of applications on plants.

1. INTRODUCTION

Agriculture, which is a primary source of food and raw materials for the population of the world, was found to be restricted by the growth of invasive plant species. Weeds manifest themselves by competing with crops for one or more plant growth factors resulting in a reduction in farm yield. Also, often times, they are usually toxic to animals and source of infections to agricultural products (Annad *et al.*, 2014). The best strategy to increase crop yields is to limit the invasion of weed plants, through a weed control and management system. Some approaches been utilized to control weeds on farm lands, of which chemical herbicide application is prominent. The evolution of modern agriculture demands high food production to the world's growing population. For this reason, agrochemicals were introduced to aid crop growth and inhibit the growth of weeds. The application of chemical herbicides as a means to curb the existence of weeds has proven to have some adverse effects on ecosystem (Boocker *et al.*, 2019).

At present, chemical control methods are widely employed to control the growth of unwanted plants due to their efficacy, including the application of synthetic pesticides (Gazziero, 2015). The constant application of herbicide has been seen to result in greater weed tolerance to the herbicides being employed (Hiwa, 2017). According to FAO (2004), the usage of chemical herbicide has resulted in the endangerment of agricultural crops. The cumulative usage of agrochemicals conceivably symbolizes a practice that has to stop due to the fact that these herbicides can make the environment toxic to various animal species (Mancini *et al.*, 2008).

In respect to the ecological and political press on environmental protection and concern, there is a need for science to make non-hazardous herbicides that will be an alternative to synthetic herbicides, without supporting the emergence of resistance (Mentzelou *et al.*, 2009). This type of pesticide, known as botanical herbicides, contains numerous chemical compounds gotten from plants that may develop novel chemicals that are capable of replacing the synthetic herbicides efficiently. The fact that botanical herbicides are less toxic and pose less risk to humans, makes them a better means of controlling weeds compared to man-made herbicides. Botanical herbicides have the ability to break down quickly in the environment, which makes them less susceptible to bioaccumulation in humans or the environment.

For some decades, the utilization of plant derived substance as environmentally safe herbicide and possible synthetic substitutes has attracted high interest from many scientists all over the world (Javaid *et al.*, 2006). The components of essential oils are many, and the antibacterial role has been reported (Okoh *et al.*, 2016). Essential oils are natural products that exhibit multifarious applications including antiviral, repellent, antibacterial, anti-inflammatory and as anticancer. They have been broadly examined as a potential pest control agent (Da Silva *et al.*, 2017). Discovery and application of these substances may lead to the creation of environmentally benign bio-herbicides (Cordeau *et al.* 2016; Cuthbertson and Murchie, 2005).

Furthermore, it has been discovered that essential oils release allelopathic substances that inhibit seed germination and may be used to combat weed germination (Schleiden *et al.*, 2019). Research has demonstrated that the inhibitory effects of essential oils on seeds depends on the plants from which they are extracted (Awojide *et al.*, 2021). According to Sadrollah *et al.* (2008), eucalyptus oil gotten from Iran showed inhibition of the germination of the weed species. Maria and Maria (2018) reported that winter savory, with carvacrol and thymol as the major constituents, and peppermint essential oil, with menthol, menthone, and iso-menthone as the major constituents, exhibited an inhibitory activity on the germination of seeds of weeds tested.

In the literature, there are several studies on the essential oil composition of *Piper umbellatum* (Kambiré *et al.*, 2019; François *et al.*, 2009). Whereas there seems to be no reported work on the herbicidal effect of *P. umbellatum* in Africa. This study aimed at appraising the herbicidal effect of *P. umbellatum* essential oil against *Z. mays*, *V. unguiculata* L and *S. lycopersicum* L. as well as ascertaining the components that could be responsible for the action, thereby assisting the local farmers to prepare local remedies that can be used in weed control.

2. METHODOLOGY

Plant Materials

The seed of *Piper umbellatum* was bought at Osogbo, Osun State, subsequent to their identification at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. It was then air-dried and ground to a powder.

Essential Oil Distillation

Hydro distillation was used to extract essential oil from the ground seed of *P. umbellatum* for 6 hours. The essential oil obtained, was air- dried and then poured inside an airtight container. It was kept at 4 °C in a refrigerator.

Gas Chromatography-Mass Spectroscopy Analysis

The analysis was performed using the Agilent 6890N instrument, which has a set of flame ionization detectors and a capillary column HP-5MS (30m height, 0.25mm internal diameter, and 0.25m film thickness). Using a mass spectrometer by Agilent Technologies, model number 5973N. The temperature was ramped up from 60 °C for 1 minute at a rate of 10 °C per minute, then from 180 °C for 1 minute at a rate of 20 °C per minute. The GC-MS's temperature was held steady at its final setting for 15 minutes. A 1:10 ratio of 1 L of the sample was neatly injected into the injector at a temperature of 270 °C. Helium (carrier gas), flowed at 1.0 ml per minute. At two scans per second, the spectrum was examined between 20 and 550 m/z. The individual components identified by mass spectrometry were compared and confirmed with literature and library both which contain Kovat's retention index for hydrocarbons in respect to C₈–C₃₂ n-alkanes.

Planting of Seedlings

Before planting, a cup of sandy loamy soil was moistened with 50 ml of water and filled with a soil sample. Five *V. unguiculat* seeds were planted, one in each cup, spaced 2 cm apart in a circular pattern. The soil was then generously sprinkled over the seeds. To enable the seedlings to flourish, a greenhouse was used (Shiv *et al.*, 2003). *Z. mays* and *S. lycopersicum* L. were planted using this method.

Herbicide Formulation

The essential oil of 5, 10, 15, and 20 µL was dissolved in 1 ml of acetone and 4 ml of deionized water to create concentrations of 1, 2, 3 and 4 ml/l, respectively, to create a liquid herbicide. The 4 ml of deionized water were mixed with 1 ml of acetone to create the control formulation.

Growth Inhibitory Test

Petri dish was soaked with cotton wool saturated with water. To soften the outer coating, the seeds were soaked in distilled water for 30 minutes to speed up growth. Each seed (10) in the petri dish received a varied concentration, and over the course of five days, the roots and shoots were measured and recorded. A control experiment was conducted, and each experiment was repeated thrice. The procedure was carried out once more using *S. lycopersicum* and *Z. mays* seeds. Equation (1) was used to calculate the percentage inhibition of the root and shoot, where A represents treated seedlings (length of the roots or shoots) and B represents control seedlings (length of the roots or shoots).

$$\text{Percentage Inhibition rate (\%)} = \frac{(B-A)}{A} \times 100 \quad 1$$

Soil Application

Ten planted plants of *S. lycopersicum* were utilized for each formulation. The amount of plants in the pot that showed signs of distress at intervals of 24 hours was measured and recorded. The essential oil formulation was put to the soil around each plant to allow the herbicide formulation to reach the root, every treatment was repeated three times. The experiment was repeated with *V. unguiculata* and *Z. mays* seeds.

Foliar Application

Ten *S. lycopersicum* L planted plants were utilized for each mixture, 2 ml of the different concentrations was spread on the plants. After 24 hours, plants exhibiting signs of distress was noted and recorded. Each test conducted inclusive of the control, was done in triplicate. The experiment was repeated with *V. unguiculata* and *Z. mays* seeds.

Statistical Analysis

IBM SPSS Statistics 20 was used to analyze the data obtained in three replicates. The differences in the values of the experiments were considered to be significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

The inhibitory effect of the essential oil from *P. umbellatum* on the root of *Z. mays* is shown in Figure 1. After 48 hours of the experiment, the first indication of root inhibition action was noted, and this was true for all graded concentrations. At a dose of 4 ml/l (31.4%), the most inhibitory activity was seen on *Z. mays* root. The varying doses' inhibitory effects grew stronger over time. Similarly, Daizy *et al.* (2004) revealed that essential oil from *Eucalyptus citriodora* delayed the sprouting of plants in exposure response dependent. In contrast to the control experiment, which exhibited no inhibitory activity at any point over the experiment's hours. The highest root growth delay of 60.6% was discovered after 144 hours.

Figure 3 depicts the outcome of the essential oil from *P. umbellatum*'s inhibitory action on the root of *V. unguiculata*. The findings showed that the root's inhibitory activity began to manifest itself after 24 hours, and varied levels of activity were detected at all graded concentrations. After 24 hours, the concentration of 4 ml/l showed 18.9% root inhibitory action, compared to 5.6% for the concentration of 1 ml/l for the roots of *V. unguiculata*. The different concentrations of the essential oil isolated from *P. umbellatum* had dose- and time-dependent root inhibitory action. For 4 ml/l concentration employed, the maximum activity was detected after 120 hours of the experiment, with a root retarding activity of 63.2%.

A test was also conducted to determine the essential oil from *P. umbellatum*'s root's ability to suppress *S. lycopersicum* growth. The experiment's findings showed root inhibitory action at all graded essential oil concentrations (Figure 5). After 48 hours, the first indication of root inhibition action was noted, and this was true for all graded concentrations. 48 hours after using a concentration of 1 ml/l, a root inhibitory activity of 12.3% was seen. It was noticed that the root inhibitory action increased as the dose rose, demonstrating that it was dose and time dependent. The formulation of the essential oils at 4 ml/l exhibited the highest activity on *S. lycopersicum* for 144 hours (60.4%).

Figures 2, 4, and 6 depict activity at various concentrations on the shoots of *Z. mays*, *V. unguiculata*, and *S. lycopersicum*, respectively. After 48 hours of the experiment, no inhibitory activities were seen on the shoots of all the plants. After 72 hours, all seeds showed the first signs of shoot inhibition, and when *P. umbellatum* essential oil at a dosage of 1 ml/l was applied to the seeds, the shoot inhibitory activities ranged from 15.2% to 100%. When 1 ml/l was utilized in 72 hours, the seed of *V. unguiculata* recorded 100% inhibitory activity of the shoot, whereas 100% suppression of the the shoots of *S. lycopersicum* and *Z. mays* were observed after 3 days with a dose 4ml/l.

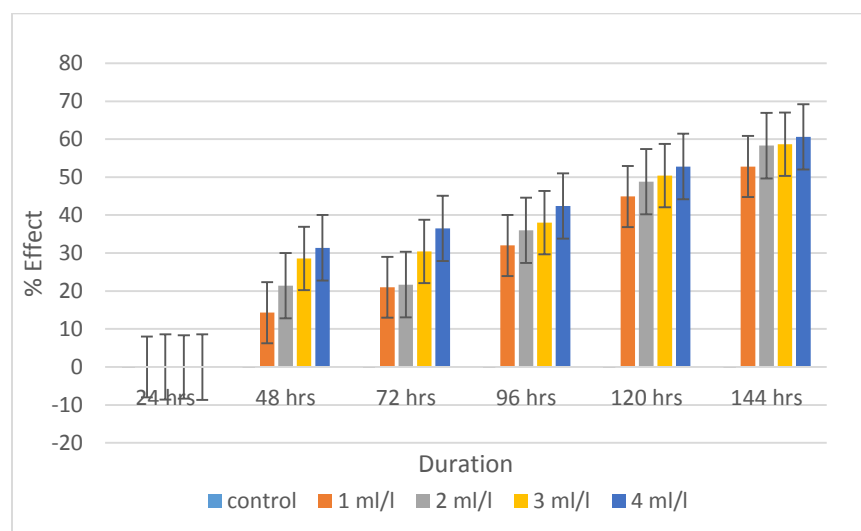


Figure 1: Effect of different concentrations on the *Z. mays* root

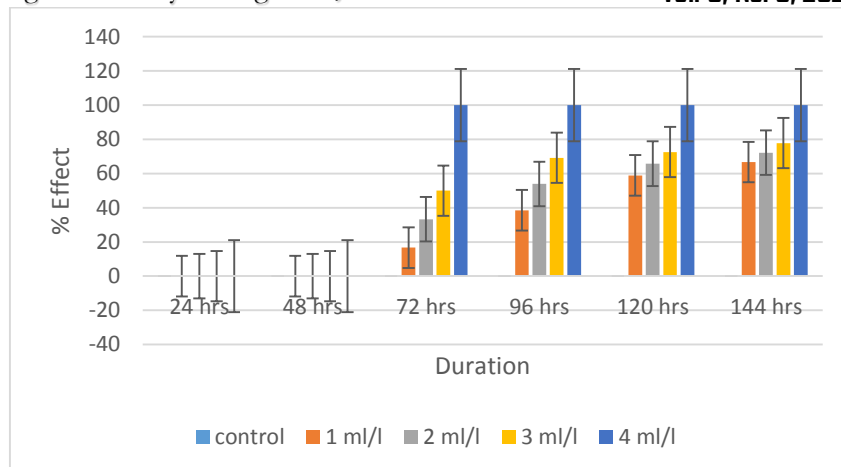


Figure 2: Effect of different concentrations on *Z. mays* shoot

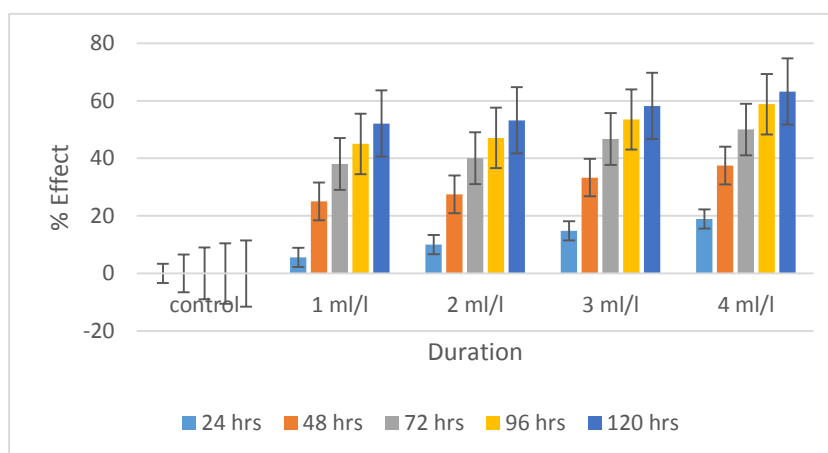


Figure 3: Effect of different concentrations on *V. unguiculata* root

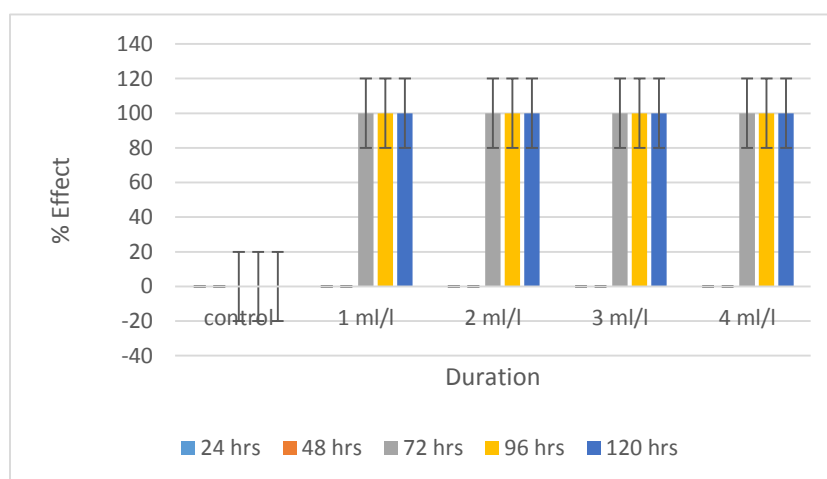


Figure 4: Effect of different concentrations on *V. unguiculata* Shoot

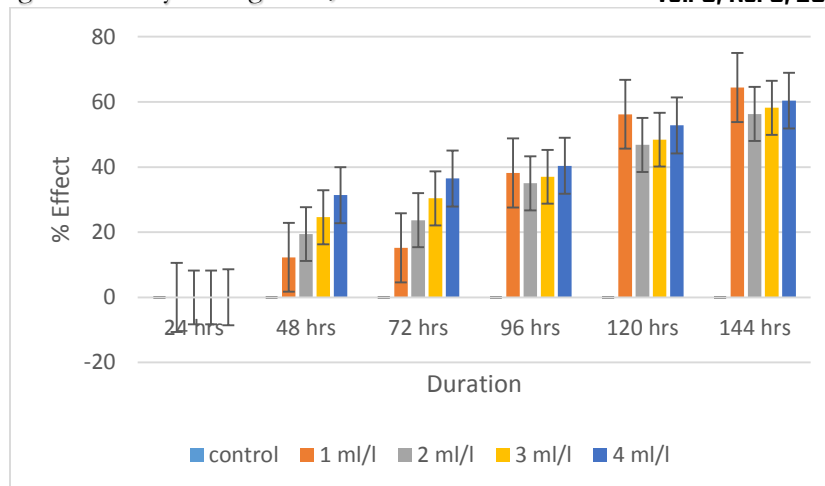


Figure 5: Effect of different concentrations on *S. lycopersicum* root

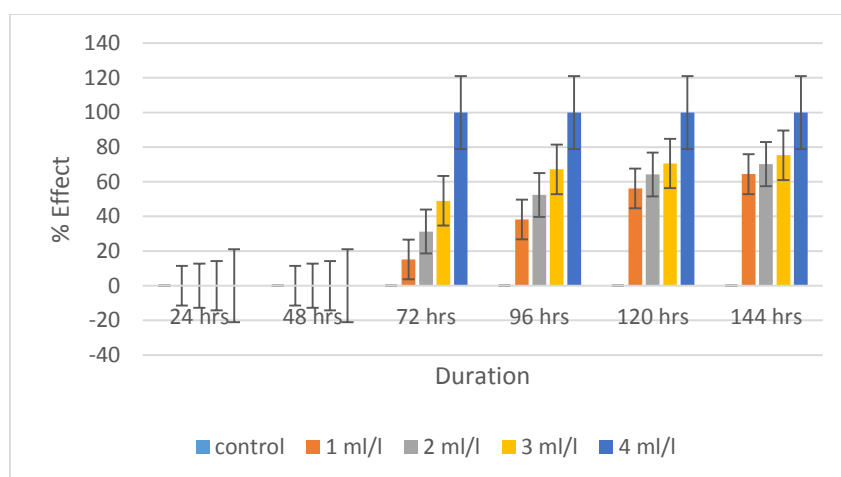


Figure 6: Effect of different concentrations on *S. lycopersicum* shoot

Figure 7 depicts the results of applying *P. umbellatum* essential oil in varying doses to the leaves of seedlings of *V. unguiculata* and *Z. mays*. The plants displayed a phototoxic impact by the discoloration of the leaves at the lowest dosage of 1 ml/l. After 24 hours, *V. unguiculata* had a 100% foliar activity, compared to 70% for *Z. mays* seedlings and 40% for *S. lycopersicum*. The application of the doses to the seedlings, resulted in yellow leaves, which is a symptom of phototoxicity. With 2 ml/l concentration, the seedlings of *V. unguiculata* also recorded 100% toxicity. However, *S. lycopersicum* seedling had the least foliar damage after 24 hours.

Figure 8 shows the essential oil of *P. umbellatum*'s root zone inhibitory effect against seedlings of the three plants. With doses of 1 ml/l, the results showed that it was 100% hazardous to the root zone of *V. unguiculata*. When a 2 ml/l dose was administered over the course of 24 hours to *Z. mays*, the root zone was completely affected this evidence by falling of the seedlings. *S. lycopersicum* was least the least effected on the root zone. The essential oil of *P. umbellatum* inhibited seed germination, when compared to the control studies, which lacked essential oil, this is similar to what Maria and Maria (2018) reported, who revealed the effect of peppermint essential oil on the germination of root and shoot with varied concentration. The results showed that *V. unguiculata* had the highest root inhibitory activity of all the seeds tested with *P. umbellatum* essential oil, while *S. lycopersicum* had the lowest activity. This was comparable

to what Maria and Maria (2018) reported, who stated that tomatoes displayed higher toxicity with peppermint essential oil than other plants. The outcome noted for the seed shoot was comparable. According to the findings (Arshad, 2009; Kritchaya, 2013), the effect of the various concentrations was more on the roots than the shoots. This may be a hint that the roots begin to show signs of growth before the shoots does.

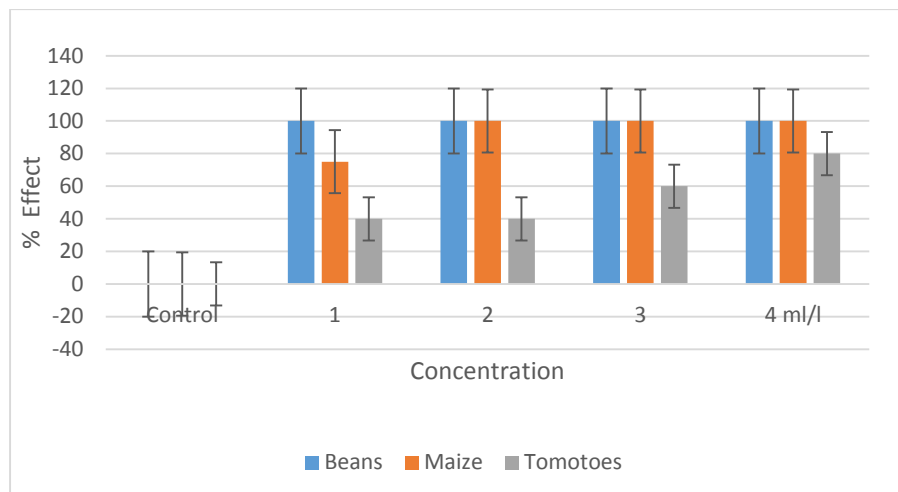


Figure 7: Foliar effect of *different concentrations* on three different plants after 24 hours of application

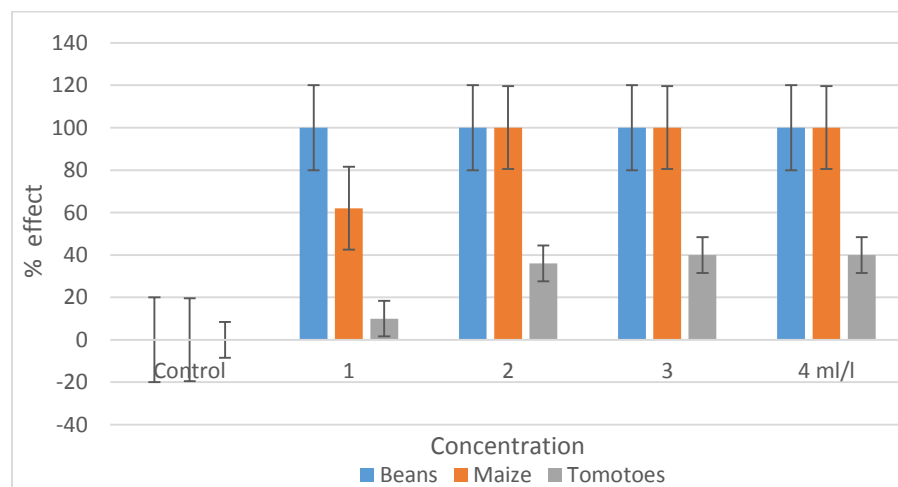


Figure 8: Root effects of different concentrations on root zone of three plants after 24 hours of application

From the results presented in Table 1, the major components observed from the GC/MS analysis of the essential oil of *P. umbellatum* are Caryophyllene (10.44%), Aromadendrene (13.74%), γ -Bisabolene (8.06%), β -Sesquiphellandrene (4.58%), Nerolidol (3.47%). This shows that sesquiterpenes are the only abundant components that abound in the essential oil that was extracted from the seed of *P. umbellatum*. Thus, the presence of sesquiterpenes as the major components present in the essential oil of *P. umbellatum* revealed the phytotoxic effect of sesquiterpenes. This result is in accordance with the study reported by Ahluwalia *et al.* (2014), who reported that essential oils rich in sesquiterpenes and essential oils rich in monoterpenes have the same inhibitory effect on the seedlings Linalool, caryophyllene, aromadendrene and germacrene were reported by Awojide *et al.* (2016).

Table 1: Major Component of the Essential oil of *P. umbellatum*

Components	Percentage composition	RI _(cal)	RI _(literature)
Caryophyllene	10.44	14101	1419.3
Aromadendrene	13.74	1429.2	1439.0
γ - Bisabolene	8.06	1523.3	1525.6
β -Sesquiphellandrene	4.58	1523.3	1530.4
Nerolidol	3.47	1540.2	1550.1

The phytotoxicity of the essential oil from the seed of *P. umbellatum* was depicted in the discoloration of the leaves of the seedlings of *V. unguiculata*, *S. lycopersicum* and *Z. mays*. A higher foliar activity was observed in *V. unguiculata* at all dose used, while *S. lycopersicum* recorded the least activity. The morphology or orientation of the leaves may be responsible for the observed discrepancy in cytotoxic effects. Due to *V. unguiculata*'s distinctive broad-sized leaves, which, in contrast to other plants, were large enough to permit the retention of the essential oil on the leaves. Observations from this study correlated with what Faria (2015) reported, essential oil applications induced shoot chlorosis as well as drooping.

4. CONCLUSION

The present research study showed that essential oil from *P. umbellatum* shows a strong phytotoxic effect on the leaves and root zone of *V. unguiculata*, *S. lycopersicum* L. and *Z. mays*. The essential oil also inhibited the growth of the root as well as the shoot of the seeds of the three plants used in this study, but the effect of the essential oil was dose and time dependent. The phytotoxicity also varied with the plant species. The biodegradability of the essential oil could make it a possible 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.

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