Phytotoxicity Activities of Essential Oil of *Pinus sylvestris* against *Zea mays*, *Solanum lycopersicum*, and *Vigna unguiculata* as Potential Bio-pesticides in Africa

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Abstract

The chemical composition of the essential oil of *Pinus sylvestris* (Scots pine), as well as its phytotoxicity action against *Zea mays*, *Vigna Unguiculata*, and *Solanum lycopersicum*, were investigated in this study. The essential oil from twigs of *P. sylvestris* was extracted using the hydro-distillation method. The major component of the essential oil was characterised by GC/MS, and the inhibitory phytotoxicity of the essential oil formulation at varying concentrations (1, 2, 3, 4, 5 mL/L) was determined by measuring the length of the shoots and the roots of tested plants. The phytotoxicity effect of the essential oil formulations on the three seeds was also determined on the foliar as well as the root site of the three seedlings by recording the number(s) of seedlings which were distressed. The major chemical constituents are mainly oxygenated monoterpenes. The compounds are Longifolene (5.45%), Borneol (6.72%), β-terpineol (14.07%), Terpinen-4-ol (21.82%) and α-terpineol (27.17%). The root and shoot inhibitory toxicity indicated that phytotoxicity was highest on the seeds of *V. unguiculata*. A varying degree of toxicity was reported on the activity of the essential oil on the leaves of the seedlings of *Z. mays*, *S. lycopersicum* and *V. unguiculata* after 24 hr. *S. lycopersicum* got the lowest level of toxicity when tested on the roots and leaves of seedlings. The seedlings of *Z. mays* and *V. unguiculata* showed 100% distress when 4 mL/L of the essential oil formulation was applied, while *S. lycopersicum* recorded 30% distress of seedlings. All phytotoxicity activities observed were time and dose-dependent. The essential oil of *P. sylvestris* showed appreciable phytotoxicity activity against the three tested plants; hence, it may be considered to be a potential bioherbicide.

Keywords: *Pinus sylvestris*, Phytotoxicity, Herbicide, Essential oil

Introduction

From time immemorial, there has been a global dependence on agriculture to feed the populace. Weeds are defined as unwanted plants that have no practical purpose and thrive in a variety of environments, particularly cultivated fields or land. Their presence in culitvated fields reduces crop yield by competing for soil, nutrients, water, and growing space with desired plants (Ismail, Lamia, Mohsen, & Bassem, 2013). Furthermore, biotic and abiotic constraints have been the major problems faced in crop production. Weeds, being among the biotic constraints, have been considered the most detrimental to agriculture production (Gharde et al., 2018; Kumar & Dwivedi, 2018; Kumar, Kumar, Naik, Yummam, & Purnima, 2018; Kumar et al., 2018).

Weed management is essential for crop protection. Of the several methods used in controlling weeds, chemical methods are preferred because of their simple application and quick action. However, the widespread and continual usage of both chemical and synthetic herbicides in agriculture has resulted in many negative consequences for the environment and the local ecology (Rassaeifar, Hosseini, Asl, Zandi, & Aghdam, 2013).

There has been an increase in the number of weeds resistant to synthetic herbicides due to their continual usage (Rassaeifar et al., 2013). Hence, the search for an eco-friendly approach to controlling weeds, particularly with herbicides derived from natural sources. Essential oils are natural blends of volatile compounds with specific medicinal characteristics such as being anti-inflammatory, antibacterial, herbicidal, antioxidant, and cancer chemoprotective, useable in insecticides as well as herbicides (Dhifi et al., 2016; Morsy, 2017; Mehdizadeh & Moghaddam, 2018). Essential oils are natural products that have become relevant due to their herbicidal activities (Sara, Mohamed, Nadjia, & Abdelkrim, 2019). Ibáñez and Blázquez (2020) reported on the phytotoxic action of various essential oils against weed seed germination, as well as their applicability in weed control methods. *Pinus sylvestris* (Scots pine) is a huge, long-lived, commercially important coniferous tree whose bark and wood can be utilized for a variety of uses. In addition, it is prized for its therapeutic and aromatic virtues, as well as for providing raw herbal materials including needles (Pini Folium), buds (Pini Gemmae), and young shoots (Pini Turiones) (Matłok, Gorzelany, Piechowiak, & Balawejder, 2020). Scots pine essential oil contains a high concentration of monoterpene hydrocarbons (Kucharska, Szymańska, Wesołowski, Bruchajzer, & Frydrych, 2018).

The antimicrobial properties of the essential oil of *P. sylvestris L.* (Scots pine) have been evaluated and documented. It has a greater insect larvicidal activity against Drosophila melanogaster (Meigen) than against other species such as *P. nigra* subsp., *P. peuce* and *P. mugo* subsp. (Mitić et al., 2018). The significant antimicrobial action may be attributed to α-pinene and β-pinene as the major constituents in the essential oil of *P. sylvestris.* It has been reported that phytotoxic action differs greatly depending on the chemical constituents of the essential oil which is determined by extrinsic and intrinsic factors such as geographic location, extraction method, drying period, temperature, and harvest time. (Ibáñez & Blázquez, 2019). The selection of *P. sylvestris* is based on the availability of the tree in most parts of Africa, while the selection of the three plants, which were *Z. mays*, *S. lycopersicum* and *V. unguiculata*, was based on (a) the differences in morphology (dicotyledon and monocotyledon), and (b). The information obtained will give a basic idea for further research on weeds with the same morphology. However, the herbicidal activity of *P. sylvestris* has been limited to reports. This study is aimed at evaluating the phytotoxicity of *P. sylvestris* against *Z. mays*, *S. lycopersicum*, and *V. unguiculata.*

Methods and Materials

Plant Materials

The Scots pine (*P. sylvestris*) twigs were obtained at Awolowo Hall, Obafemi Awolowo University, Ife, Osun State, Nigeria. The twig was identified with a herbarium specimen, IFE-17939, at the Botany Department of Obafemi Awolowo University. The twigs were air-dried and cut into smaller bits.

Essential Oil Distillation

The *P. sylvestris* twigs were ground into powder and subjected to hydro-distillation of the essential oil extract for 6 hr using a modified Clevenger-type apparatus. Anhydrous sodium sulphate was used to dry the extract. Before analysis, the extracted essential oils were kept refrigerated in sealed containers at 4°C

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

An Agilent 6890N equipped with a flame ionization detector and Capillary column HP-5MS (30m by 0.25mm by 0.25μm) was used to perform the GC-MS analysis. An Agilent 5973N mass spectrometer was used to identify the essential oil's components. The GC was set for 1 min at a temperature of 60°C. It was then ramped for 1 min at 10°C min^{-1} to 180°C and finally heated to 280°C and held for 15 min at 20°C min^{-1}. The temperature of the injector was kept at 270°C while 1 μL of the essential oil was injected. The carrier gas, which was helium, flowed at a rate of 1.0 mL/ min. Mass spectra were acquired over the mass range 30-400 Da, ionization voltage 70 eV; ion source temperature 200°C. Spectra were scanned from 20 to 550 m/z. The compounds were identified by comparing their Kovat's retention index with hydrocarbons relative to n-alkanes and mass spectra with reference samples or data already accessible in the NIST 2008, mass spectral library and the literature.

Planting of seedlings

Plastic cups were filled to 2 cm from the rim with sandy loamy soil, and 50 mL of water was added. In each cup, five *Vigna unguiculate* seeds were planted in a circular configuration, 2 cm apart, and then covered with soil. The seedlings were planted and then placed in a greenhouse to allow them to grow enough leaves (Shiv et al., 2003). *S. lycopersicum L.* and *Zea mays* seedlings were planted using the same method.

Herbicidal activity

Herbicide formulation

Several essential oil samples, 5, 10, 15 and 20 μL, were dissolved in 1 mL of acetone (emulsifier) and 4 mL of deionized water to make a liquid herbicide formulation with concentrations of 1, 2, 3 and 4 mL/L. 1 mL acetone in 4 mL deionized water was used to create a control formulation.

Growth inhibitory test

Ten seeds of *S. lycopersicum*, *Z. mays* and *V. unguiculata* were utilized for each mixture of herbicide formulation. For *V. unguiculate*, the seeds were steeped in water before being placed on cotton wool in a petri dish (to soften the outer covering and facilitate rapid germination, the seeds were first immersed in water for two hr.). Each seed in the petri dish was administered the essential oil formulation for four days, and the length of root and shoot growth was measured and documented daily. For each test run, a controlled experiment was carried out. Each treatment was reproduced three times with a complete set of controls. Seeds from were used in the experiment. The following equation was used to compute the root and shoot length inhibition rate:

$$\text{Percentage rate of inhibition } (\%) = \frac{A-B}{A} \times 100$$

where B represents the shoots/roots length of the treated seedlings, and A represents the shoots/root length of the untreated, control seedlings.

Soil application

Ten seeded *V. unguiculata* plants were utilized for each formulation. The essential oil formulation was sprayed on the soil around each plant to allow the herbicide formulation to reach the root, and the number of plants in the pot that showed signs of distress were counted and recorded every 6 hr for 48 hr. For each test completed, a control experiment was carried out. Each treatment was reproduced three times with a complete set of controls. The same experiment was carried out on the seeds of *S. lycopersicum and Z. mays* using 10 seeds of each.

Foliar application

Ten seeded *V. unguiculata* plants were used in each formulation. The essential oil formulation was sprayed on the plants in the pot to allow the herbicide formulation to reach the leaves, and the number of plants in the pot that showed distress was recorded every 6 hr for 48 hr. The amount of solvent employed was determined by the leaf's ability to retain the solution. Each test included a control experiment. The experiment was repeated twice. Seeds of *S. lycopersicum and Z. mays* were also subjected to the same experiment.

Statistical analysis

The experiments were carried out and repeated twice, and the analysis of the data was done using SPSS statistics to perform analysis of variance (ANOVA). Differences were considered to be significant at $p \leq 0.05$.

Results

Chemical compositions of *P. sylvestris* essential oil

The major chemical constituents of the essential oil of *P. sylvestris* are shown in Table 1. These chemical were Borneol (6.72%), β-terpineol (14.07%), Terpinen-4-ol (21.82%), α-terpineol (27.17%), which can be grouped as oxygenated monoterpenes, and Longifolene (5.45%) as sesquiterpene hydrocarbon.

Table 1 Major constituents of essential oil of *P. sylvestris*

COMPOUNDS	%	RI ($_{cal}$)	RI ($_{literature}$)
Terpinen-4-ol	21.82	1152.3	1164.5
β -terpineol	14.07	1119.2	1129.3
Borneol	6.72	1145.1	1153.2
α -terpineol	27.17	1170.3	1175.6
Longifolene	5.45	1400.2	1404.0

RI ($_{literature}$) represents the retention indices as reported by Babushok, Linstrom, & Zenkevich, 2011. Retention indices of components for dimethylsilicone stationary phase.

Phytotoxicity effect of the essential oil formulations

The root inhibitory activities of *P. sylvestris* essential oil formulations against *Z. mays*, *S. lycopersicum* and *V. unguiculata* are shown in Table 2-4. The inhibitory activity was observed to be time-dose dependent, indicating an increase in inhibitory effects with increasing concentrations of essential oil with extended times of application. *P. sylvestris* showed inhibitory activity against *Z. mays* and *S. lycopersicum* at all applied doses (1, 2, 3, and 4 mL/L). The lowest inhibition rate for *Z. mays* was observed at a concentration of 1 mL/L (28.57%) after one day, while the highest was obtained at a concentration of 4 mL/L (84.62%) after four days. An inhibition rate of 13.60% was observed after one day for *S. lycopersicum* at a concentration of 1 mL/L. The highest inhibitory activity was obtained at a concentration of 4mL/L (60.62%) after four days of the experiment. For *V. unguiculate*, the lowest inhibition rate was observed at a concentration of 1 mL/L (14.60%) after the first day, while there was complete/ maximum inhibitory activity (100%) at all concentrations (1, 2, 3, 4 and 5 mL/L) after four days of the experiment. The control experiment void of the essential oil showed no inhibitory activity throughout the experiment.

Table 2 Percentage inhibitory activity of *P. sylvestris* essential oil on *Z. mays* root

Duration	Concentration (mL/L)				
Time(Days)	1	2	3	4	Control
1	28.57±5.80b	35.71±5.80c	40.00±10.02d	52.57±5.80b	0.00±0.00a
2	31.78±5.80b	37.33±10.00c	45.33±5.80c	57.44±5.80d	0.00±0.00a
3	40.00±10.00b	50.00±10.00c	52.00±5.80c	65.00±5.80e	0.00±0.00a
4	61.54±10.00c	61.00±5.80b	73.08±5.80d	84.62±5.80e	0.00±0.00a

The data 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 Percentage inhibitory activity of *P. sylvestris* essential on *S. lycopersicum* root

Duration	Concentration (mL/L)				
Time(Days)	1	2	3	4	Control
1	13.60±5.80^{b}	20.71±5.80^{c}	25.00±10.02^{d}	30.57±5.80^{b}	0.00±0.00^{a}
2	15.78±5.80^{b}	22.33±10.00^{c}	30.33±5.80^{c}	35.44±5.80^{d}	0.00±0.00^{a}
3	26.00±10.00^{b}	35.00±10.00^{c}	40.00±5.80^{c}	43.00±5.80^{e}	0.00±0.00^{a}
4	40.54±10.00^{c}	43.00±5.80^{b}	50.10±5.80^{d}	60.62±5.80^{e}	0.00±0.00^{a}

The data 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 Percentage inhibitory activity of *P. sylvestris* essential oil on *V. unguiculata* root

Duration	Concentration(mL/)				
Time(Days)	1	2	3	4	Control
1	14.60±5.80^{b}	86.00±10.00^{c}	100.00±0.00^{d}	100.00±0.00^{e}	0.00±0.00^{a}
2	53.00±10.00^{c}	100.00±0.00^{e}	100.00±0.00^{d}	100.00±0.00^{e}	0.00±0.00
3	100.00±0.00^{d}	100.00±0.00^{e}	100.00±0.00^{d}	100.00±0.00^{e}	0.00±0.00
4	100.00±0.00^{d}	100.00±0.00^{e}	100.00±0.00^{d}	100.00±0.00^{e}	0.00±0.00

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

The shoot inhibitory activity of *P. sylvestris* against *Z. mays* is shown in Table 5, *V. unguiculata* in Table 6, and *S. lycopersicum* in Table 7. There was no inhibitory activity on the shoots of *Z. mays*, *V. unguiculata* and *S. lycopersicum* after the first two days. The initial inhibitory effect on *Z. mays* was observed after three days at a concentration of 1 mL/L (36.32%). As the concentration increased from 1 mL/L to 4 mL/L, 68% inhibition was observed. The highest inhibitory activity was obtained at a concentration of 4 mL/L (89.29%) after four days of the experiment. Initial and maximum inhibitory activity (100%) was obtained after three days of the experiment at all concentrations (1, 2, 3, 4, and 5mL/L) against *V. unguiculata*. Whereas, the first sign of inhibitory activity against *S. lycopersicum* was observed at a concentration of 1 mL/L (36.32%) after three days of experiment with the highest inhibitory effect at a concentration of 4 mL/L (87.29) after four days of experiment. Therefore, the inhibitory activity of the essential oil of *P. sylvestris* against the shoots of each species

is time-dose dependent. The control experiment showed no inhibitory effect at all against *Z. mays*, *V. unguiculata* and *S. lycopersicum*.

Table 5 Percentage inhibitory activity of *P. sylvestris* essential oil on *Z. mays* shoot

Duration	**Concentration (mL/L)**				
Time(Days)	**1**	**2**	**3**	**4**	**Control**
1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
3	36.32±5.80[b]	57.90±5.80[c]	65.42±5.80[e]	68.00±5.80[d]	0.00±0.00[a]
4	69.64±5.80[b]	87.50±5.80[c]	89.29±5.80[e]	89.29±5.80[e]	0.00±0.00[a]

The data 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 Percentage inhibitory activity of *P. sylvestris* essential oil on *V. unguiculata* shoot

Duration	Concentration(mL/)				
Time(Days)	1	2	3	4	Control
1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
3	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]	0.00±0.00[a]
4	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]	100.00±0.00[b]	0.00±0.00[a]

The data 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 Percentage inhibitory activity of *P. sylvestris* essential oil on *S. lycopersicum* shoot

Duration	**Concentration (mL/L)**				
Time(Days)	**1**	**2**	**3**	**4**	**Control**
1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
2	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
3	36.32±5.80[b]	56.90±5.80[c]	64.42±5.80[e]	68.40±5.80[d]	0.00±0.00[a]
4	68.64±5.80[b]	84.50±5.80[c]	86.29±5.80[e]	87.29±5.80[e]	0.00±0.00[a]

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

The graphical representation of the percentage root zone inhibitory activity of the essential oil of *P. sylvestris* against *Z. mays*, *V. unguiculata* and *S. lycopersicum* is depicted in Figure 1. At all graded concentrations (1, 2, 3, and 4 mL/L), complete root toxicity at 100% was observed for *Z. mays* within 24 hr of application. *V. unguiculata* showed 90% root toxicity at the very least concentration of 1 mL/L within 24 hr of application. *S. lycopersicum* had the least root zone toxicity at all graded concentrations. At 1 mL/L, the root zone toxicity was recorded as 100%, 90% and 0% for *Z. mays*, *V. unguiculata* and *S. lycopersicum* respectively. The greatest distress was observed in *Z. mays* and *V. unguiculata* while *S. lycopersicum* showed the least distress.

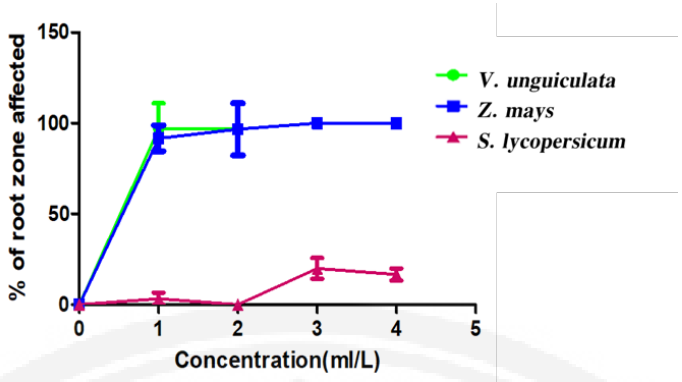

Figure 1 Percentage root zone inhibitory activity of *P. sylvestris* essential oil on *V. unguiculata*, *Z. mays* and *S. lycopersicum* after 24 hr

Figure 2 illustrates the graphical representation of the foliar activity of the essential oil of *P. sylvestris* on the leaves of *Z. mays*, *V. unguiculata* and *S. lycopersicum*. Phototoxic effect on the leaves of the seedlings of *Z. mays* and *V. unguiculata* by decolouration was evident at the least concentration of 1mL/L at 100% and 90% respectively within 24 hr of application. Whereas no phototoxic effect on the seedlings of *S. lycopersicum* was recorded at a concentration of 1 mL/L. Maximum foliar effect (100%) on the seedlings was obtained at a concentration of 4 mL/L for *Z. mays* and *V. unguiculata*. The least foliar effect (20%) on the seedlings was observed for *S. lycopersicum* at a concentration of 2 mL/L within 24 hr of application.

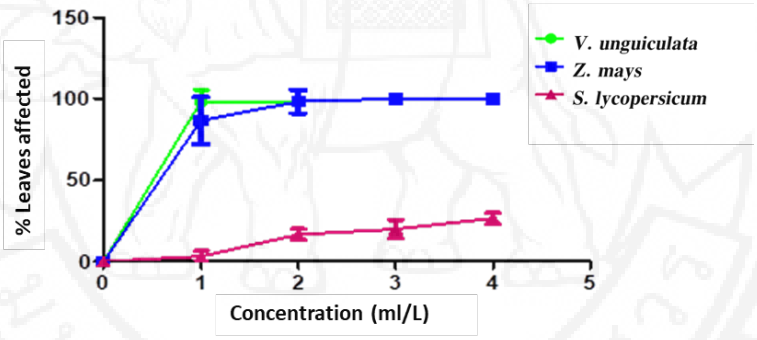

Figure 2 Percentage foliar activity of *P. sylvestris* essential oil on *V. unguiculata*, *Z. mays* and *S. lycopersicum* after 24 hr

Discussion

The essential oil of *P. sylvestris* had a dose-dependent phytotoxic action against the germination of seeds of the tested plants when compared with the control experiments. This result was in agreement with the report of Ibáñez and Blázquez (2020), who recorded the dose-dependent phytotoxic activity of oregano essential oil against germination of seeds of cucumber and tomato. Uremis, Arslan, and Sangun (2009) reported the herbicidal activities of the essential oils of *Ocimum basilicum*, *Salvia officinalis*, *Lavender angustifolia*, *Melissa officinalis*, and *Thymus vulgaris* against three weeds. The results indicated that the germination inhibition rate of the essential oils increased with increasing concentrations, which is the same as reported in this present research. The essential oils demonstrated varied activities on the different weeds tested against some essential oils, with Thymus vulgaris essential oil having the most inhibitory effect on the germination of *Xanthium strumarium* (Uremis et al., 2009). This is similar to the finding of this work, where *Z. mays* recorded the least

(65.0%) root inhibitory activity with a dose of 4 mL/L after three days, while 100% inhibition was recorded for *V. unguiculata* and *S. lycopersicum*.

The highest foliar activity of the essential oil of *P. sylvestris* was observed on the leaves of *Z. mays* and *V. unguiculata* whereas *S. lycopersicum* exhibited the least activity. This matched the findings of Awojide, Oyewole, Abiona, & Agbaje. (2021), who reported the least foliar activity on the *S. lycopersicum* compared to *Z. mays* and *V. unguiculata* with the essential oil of *Piper nigrum*. The same trend was reported by Vishwakarmaa and Mittala (2014).

In this present study, the major chemical constituents of the essential oil of *P. sylvestris* were a group of oxygenated monoterpenes (Borneol, β-terpineol, Terpinen-4-ol, α-terpineol) and a sesquiterpene hydrocarbon, Longifolene. Awojide et al. (2023) reported the presence of Terpinen-4-ol (11.28%), Longifolene (8.84%), isobornel (11.82%) and fenchol (10.45%) in the crude essential from the twig of *P. sylvestris*. α-terpineol (27.17%), which had the highest constituent of its reported effects on *Sonchus. arvensis*, *Erica vesicaria* and *Scorpiurus muricatus*, are said to have phytotoxicity effects (De Martino et al., 2010; Gichi et al., 2016). Borneol was reported as a major constituent in the essential oil of *Rosmarinus officanalis* L. and it is attributed to the phytotoxic potential of plants (Angelini *et al.*, 2003). Terpinen-4-ol (21.82%) is one of the major components of *Drimys winterii* which is also regarded as being responsible for its herbicidal activities (Verdeguer, 2011). The higher percentage of oxygenated monoterpenes present in the essential oil of *M. myristica* was responsible for the phytotoxic activities observed on the seed as well as the seedlings. Oxygenated monoterpenes have been reported to exhibit higher herbicidal properties (Ibáñez & Blázquez, 2019). In a study conducted by Kordali, Cakir, and Sutay (2007) reported high phytotoxic activity of oxygenated monoterpenes such as β-citronellol, nerol and Terpinen-4-ol was demonstrated, in which they demonstrated a total inhibitory effect on seed growth and seed germination. The presence of a higher percentage of oxygenated monoterpene in the essential oil of *P. sylvestris* could be responsible for its phytotoxic activity.

Conclusion and Suggestions

From this study, it could be inferred that the essential oil of *P. sylvestris* showed great herbicidal activity on *Z. mays*, *V. unguiculata*, and *S. lycopersicum*. It could be deduced from the results that the phytotoxic effect is time-dose dependent and plant-species-dependent. The availability of *P. sylvestris* trees in Africa makes them accessible to local farmers. The method of extraction of the essential oil as well as its application, which is water dependent, can be easily achieved. Therefore, the essential oil of *P. sylvestris*, as a natural herbicide, would help reduce the adverse effects of the synthetic herbicide on human health and the environment.

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