

Research Article

Allelopathic Effects of *Sphaeranthus suaveolens* on Seed Germination and Seedling Growth of *Phaseolus vulgaris* and *Oryza sativa*

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Received 30 September 2020; Revised 6 January 2021; Accepted 4 February 2021; Published 15 February 2021

Academic Editor: Othmane Merah

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Weeds with allelopathic effect have been reported to cause significant damage in agriculture particularly in smallholder farming systems. This study assessed the allelopathic effects of different concentrations of crude extract of a noxious weed *Sphaeranthus suaveolens* on seed germination and seedling growth of *Phaseolus vulgaris* and *Oryza sativa* by examining germination, seedling height, and total chlorophyll content after seven and fourteen days of crude extract treatment, respectively. Results showed that seed germination and seedling growth were significantly ($p < 0.001$) decreased with increase in concentration of crude extract, signifying concentration dependency. Highest concentration (100%) of *S. suaveolens* crude extracts resulted in 90% and 100% inhibition of *P. vulgaris* and *O. sativa* seed germination, respectively. Chlorophyll content, fresh weight, and root and shoot length of both *P. vulgaris* and *O. sativa* were also significantly ($p < 0.001$) affected by highest concentration (100%) of *S. suaveolens* crude extracts. Results from this study suggest that the extract of *S. suaveolens* contains water-soluble allelochemicals which significantly reduce growth and productivity of *P. vulgaris* and *O. sativa*.

1. Introduction

Weed invasion is becoming a major challenge in agricultural sector worldwide particularly in smallholder farming systems [1, 2]. Weeds have been reported to significantly affect crop production by competing for light, water, nutrients, and space thereby threatening the economic growth and food security of smallholder farmers [3–6]. Additionally, these unwanted plants have been observed to host insect pests and diseases [7–9] as well as disrupting and interfering with natural interactions by displacing native species, distracting pollinators, and other insects that are beneficial in the smallholder farming systems [10–12].

Most weeds have been alleged to possess allelopathic effects which play an important role in their invasion success

[13–15]. Allelopathy is a phenomenon, whereby one plant influences the growth of biological systems, including microorganisms, by the release of chemical compounds into the environment [16–18]. The allelopathic effects are the result of plant's secondary metabolites known as allelochemicals, which are usually byproducts of the principal metabolic pathways in plants [19–21]. These allelochemicals can be found in the leaves, stem, flowers, fruits, and roots [22].

Plants with allelopathic properties have been observed to significantly affect the growth and development of other neighboring plants by inhibiting seed germination, causing soil infertility and nutrient imbalance as well as limiting the microbial population in the soil [23–25]. Due to these effects, allelopathy has become a research hotspot for making

comprehensive analysis about the mechanism of weed invasions and possibilities of utilizing these naturally occurring phytochemicals in managing weeds and insect pests in agricultural ecosystems [26, 27].

Common bean (*Phaseolus vulgaris*) and rice (*Oryza sativa*) are among important food and income generating crops globally [28]. The two crops have been reported as the principal source of protein and main calorie supply to a significant portion of the households in Africa and globally at large [29–31]. Despite the importance of the two crops in the agricultural sector and livelihood of most smallholder farmers, yields are generally low with the average revolving around 990 kg/ha for *P. vulgaris* and 2400 kg/ha for *O. sativa* [32]. The potential yields under favorable conditions are estimated to be around 1500–3000 kg/ha for *P. vulgaris* and 2500–4000 kg/ha for *O. sativa* [33]. Among the reasons behind this low yields are heavy infestation from weeds, insect pest attacks, and poor crop management skills such as late weeding [7, 34, 35].

Sphaeranthus suaveolens is a spreading weed from the family Asteraceae, commonly found in swampy and cultivated farmlands [36, 37]. A heavy infestation of this weed results in adverse effects on the growth of adjacent plants [38]. It has also been observed that *S. suaveolens* has an ability to suppress crops in a wide range over a short period of time [39]. Additionally, significant portion of common bean and rice smallholder farmers reported major loss due to *S. suaveolens* infestations [7]. Despite of these tragic losses, the allelopathic effects of *S. suaveolens* to *P. vulgaris* and *O. sativa* have not yet been studied or reported. Understanding these effects could considerably improve the *S. suaveolens* management in farmlands and significantly reduce its effects on crop productivity. The present study was carried out to evaluate the allelopathic effects of *S. suaveolens* using different aqueous extract concentrations on germination and seedling growth of *P. vulgaris* and *O. sativa* crops under laboratory and screen house conditions.

2. Materials and Methods

2.1. Seed Preparation and Treatment. Seeds of *P. vulgaris* and *O. sativa* were collected from Selian Agricultural Research Institute (SARI) in Arusha, Tanzania, in June of 2019. Before the experiment, the seeds were air dried and stored in plastic bags. Seed viability of both plants was determined by the germination test [40], in which all the 20 seeds (100%) for each crop (10 *P. vulgaris* and 10 *O. sativa* seeds) that were selected randomly from a seed stock and planted in a Petri dish lined with cotton wool in early September 2019 germinated. Seeds were later washed using tap water and sterilized with 5 % NaOCl for 2 min and then rinsed with distilled water before planting.

2.2. Crude Extract Preparation. Freshly matured plants of *S. suaveolens* were collected from Arumeru and Moshi rural districts, Tanzania, between June and July 2019. The plants were shade dried under room temperature for 14 days, grounded into powder using a grinder, and stored in plastic

containers before the experiments. Extracts were prepared according to Ngondya et al. [41] with few modifications as follows: 100 g of *S. suaveolens* powder was soaked separately in 1 liter of distilled water and left for 72 h. Afterwards, crude extracts were filtered using Whatman filter paper no. 1 to obtain a final volume of 1 liter each. Both crude extracts (ml) were diluted with distilled water (ml) in the ratio of 25 : 75, 50 : 50, 75 : 25, and 100 : 0 (extract : distilled water) to obtain different concentrations of 25%, 50%, 75%, and 100%. The diluted extracts were kept in the refrigerator at 4°C.

2.3. Laboratory Experiment. The effects of *S. suaveolens* crude extracts on the seed germination, seedling height, and leaf chlorophyll content of *P. vulgaris* and *O. sativa* were studied using a completely randomized design (CRD) from October to November 2019. Ten seeds of each crop (*P. vulgaris* and *O. sativa*) were placed in each of the five Petri dishes (each with the surface area of 70.8 cm²) lined with cotton wool. Each Petri dish was moistened once a day with 10 ml of different concentration treatments, i.e., 0% (for control) and 25%, 50%, 75%, and 100% (for *S. suaveolens* crude extracts). Each treatment was replicated three times. Seeds were observed every day under the 12 h dark and 12 h light conditions. Number of germinated seeds was recorded and counted for 7 days for *P. vulgaris* and 14 days for *O. sativa*. Seedlings were harvested and fresh weight, seedling height, and leaf total chlorophyll content were determined for each germinated seedling. The entire experiment was repeated three times.

2.4. Screen House Experiment. The effects of crude extracts of *S. suaveolens* on the seed germination, seedling height, leaf total chlorophyll content, and fresh and dry weight of *P. vulgaris* and *O. sativa* crops were studied using a completely randomized design in a screen house from October to November 2019. Six seeds for each crop (*P. vulgaris* and *O. sativa*) were placed each in five pots with the surface area of 763.8 cm². The pots were then moistened on daily basis with 100 ml of different concentration treatments (25%, 50%, 75%, and 100%) of *S. suaveolens* crude extracts and distilled water for the control. Each treatment was replicated three times. Seeds were observed every day and the number of germinated seeds were recorded and counted for 7 days for *P. vulgaris* and 14 days for *O. sativa*. Thereafter, seedlings were harvested and fresh weight, seedling height, and leaf total chlorophyll content were determined for each germinated seedling. Similar to the laboratory experiment, this experiment was also repeated three times.

2.5. Chlorophyll Content Determination. Leaf chlorophyll of the *P. vulgaris* and *O. sativa* seedlings was extracted according to Hiscox and Israelstam (1978) with some modifications: 50 mg of each crop (*O. sativa* and *P. vulgaris*) fresh leaves of 2.25 cm² surface area were immersed in 4 ml of dimethyl sulfoxide (DMSO) and incubated at 65°C for 12 h. The extract was transferred to glass cuvettes for

absorbance determination. The absorbance of blank liquid (DMSO) and samples were determined under 2000 UV/VIS spectrophotometer (UNICO®) at 645 and 663 nm (Hiscox and Israelstam, 1978), and the leaf total chlorophyll content (Chl) was calculated according to Arnon (1949) using the following equation:

$$\text{total Chl} = 0.0202A_{663} + 0.00802A_{645}, \quad (1)$$

where A_{663} and A_{645} are absorbance readings at 663 and 645 nm, respectively.

2.6. Data Analysis. Data on allelopathic effects of *S. suaveolens* on seed germination and seedling growth (shoot length, root length, fresh weight of shoot, and fresh weight of root and chlorophyll content) of *P. vulgaris* and *O. sativa* were compared using one-way ANOVA. The normality and homogeneity of variance were verified using Shapiro–Wilk test and Levene’s test, respectively. Fishers LSD test was used to compare the significance differences between the group means. The statistical software used for all tests was Origin (version 2018b) at a significance level of 5%.

3. Results

3.1. Seed Germination. Generally, higher concentrations (75% and 100%) of *S. suaveolens* in both laboratory and screen house experiments were effective in suppressing both *P. vulgaris* and *O. sativa* seeds germination. The germination of *P. vulgaris* and *O. sativa* seeds was delayed at higher concentrations (75% and 100%) compared with the negative control (0%) and lower concentrations (25%) of the *S. suaveolens* crude extract. The mean percentage germination under 0% concentration (negative control) was 100% for *P. vulgaris* and 90% for *O. sativa* in the laboratory, as compared with 100% for both *P. vulgaris* and *O. sativa* in the screen house experiment. Additionally, under higher concentration (100%), the mean percentage germination for *P. vulgaris* was 10% and 0% in laboratory and screen house experiments, respectively, while for *O. sativa*, it was 0% in both experiments (Table 1). In general, the seed germination for both *P. vulgaris* and *O. sativa* decreased significantly ($p < 0.001$) with the increase in the concentration of *S. suaveolens* crude extract (Table 1).

3.2. Shoot Length. Shoot length of *P. vulgaris* and *O. sativa* seedlings sprayed with *S. suaveolens* concentrations differed significantly in the laboratory ($F_{(4, 15)} = 56.64$, $p < 0.0001$, and $F_{(4, 15)} = 52.65$, $p < 0.0001$) and screen house ($F_{(4, 15)} =$, $p < 0.0001$, and $F_{(4, 15)} = 52.65$, $p < 0.0001$) experiments, respectively (Figures 1 and 2). Mean (\pm SE) seedling lengths of *P. vulgaris* and *O. sativa* in 0% treatments (16 ± 1 cm and 8 ± 1 cm) were 5 and 8 times longer than the ones in 100% treatments (3 ± 0 cm and 0 ± 0 cm) in both laboratory and screen house experiments. In general, the shoot length for *P. vulgaris* and *O. sativa* seedlings decreased significantly ($p < 0.001$) with the increase in concentration of *S. suaveolens* crude extract in both the laboratory and screen house experiments.

3.3. Root Length. The root length of *P. vulgaris* and *O. sativa* seedlings sprayed with *S. suaveolens* crude extract concentrations differed significantly in both laboratory ($F_{(4, 15)} = 165.89$, $p < 0.001$, and $F_{(4, 15)} = 34.66$, $p = 0.001$) and screen house ($F_{(4, 15)} = 10.37$, $p < 0.001$, and $F_{(4, 15)} = 47.55$, $p < 0.001$) experiments (Figures 3 and 4). At higher concentration (100%) of *S. suaveolens* crude extract, the mean root length (\pm SE) in *P. vulgaris* (0 ± 0.1 cm and 2 ± 0.3 cm) and *O. sativa* (0 ± 0 cm) seeds was significantly reduced ($p < 0.001$) as compared with lower (0%) concentrations (8 ± 0.4 cm and 7 ± 0.9 cm) for *P. vulgaris* and (3 ± 0.5 cm and 4 ± 0.5 cm) *O. sativa* in both laboratory and screen house experiments, respectively (Figures 3 and 4). The root length for *P. vulgaris* and *O. sativa* seedlings in both laboratory and screen house experiments was significantly reduced ($p < 0.001$) as the concentration of *S. suaveolens* crude extract increased.

3.4. Fresh Weight of Roots and Shoots. The average fresh weight of roots (FWR) for *P. vulgaris* and *O. sativa* differed significantly with *S. suaveolens* treatment in both laboratory ($F_{(4, 15)} = 284.23$, $p < 0.001$, and $F_{(4, 15)} = 30.88$, $p < 0.0009$) and screen house ($F_{(4, 15)} = 435.35$, $p < 0.001$, and $F_{(4, 15)} = 92.71$, $p < 0.001$) experiments. The fresh weight of shoots (FWS) also differed significantly among tested crops in both laboratory ($F_{(4, 15)} = 399.39$, $p < 0.001$, and $F_{(4, 15)} = 59.12$, $p < 0.0003$) and screen house ($F_{(4, 15)} = 504.51$, $p < 0.001$, and $F_{(4, 15)} = 301.13$, $p < 0.001$) experiments. Seedlings treated with higher concentrations in both tested crops were observed to have lower fresh weights than those treated with lower concentrations in both laboratory and screen house experiments (Figures 5–8).

3.5. Total Chlorophyll Content. Total leaf chlorophyll content of *P. vulgaris* and *O. sativa* seedlings differed significantly in both laboratory ($F_{(4, 15)} = 21.53$, $p < 0.00004$, and $F_{(4, 15)} = 3.81$, $p < 0.002$) and screen house ($F_{(4, 15)} = 18.38$, $p < 0.00001$, and $F_{(4, 15)} = 71.96$, $p < 0.00001$) experiments under *S. suaveolens* crude extract treatments (Figures 5 and 6). In general, the seedlings of both tested plants (*P. vulgaris* and *O. sativa*) treated with higher (100%) concentration of *S. suaveolens* crude extracts had lower total chlorophyll content than those sprayed with lower (0%) concentrations in both laboratory and screen house experiments (Figures 9 and 10).

4. Discussion

This study revealed that the crude extract of *S. suaveolens* significantly reduced seed germination of *P. vulgaris* and *O. sativa*. This suggests that *S. suaveolens* possess water-soluble allelochemicals which showed inhibitory effects on the two tested crops. Moreover, at higher concentrations (75% and 100%), the *S. suaveolens* extracts showed maximum inhibition in the germination of *P. vulgaris* and *O. sativa*. These results are in agreement with the study conducted by [42, 43] on the allelopathic effects of various weeds on seed germination of rice and beans where germination was reduced to 20% and 6%, respectively. The

TABLE 1: Mean percentage germination (\pm SE) of *P. vulgaris* and *O. sativa* seeds (after 7 and 14 days, respectively) per treatment of *S. suaveolens* extracts of treatment in a laboratory and screen house experiments.

Concentration (%)	Laboratory experiment		Screen house experiment	
	<i>P. vulgaris</i>	<i>O. sativa</i>	<i>P. vulgaris</i>	<i>O. sativa</i>
0	100 \pm 0.2 ^a	90 \pm 0.3 ^a	100 \pm 0.1 ^a	100 \pm 0 ^a
25	80 \pm 0.4 ^b	90 \pm 0.2 ^a	83 \pm 0.4 ^b	83 \pm 0.3 ^a
50	70 \pm 0.4 ^c	50 \pm 0.6 ^b	50 \pm 0.4 ^c	33 \pm 0.4 ^b
75	20 \pm 0.4 ^d	10 \pm 0.3 ^c	17 \pm 0.2 ^d	17 \pm 0.3 ^c
100	10 \pm 0.2 ^e	0 \pm 0.2 ^c	0 \pm 0.2 ^e	0 \pm 0 ^c
F-statistics	$F_{(4, 15)} = 142^*$	$F_{(4, 15)} = 140^*$	$F_{(4, 15)} = 53^*$	$F_{(4, 15)} = 144^*$

Values with different superscript letter(s) in the same column are significantly different by Fisher LSD at $p = 0.05$. * $P < 0.001$.

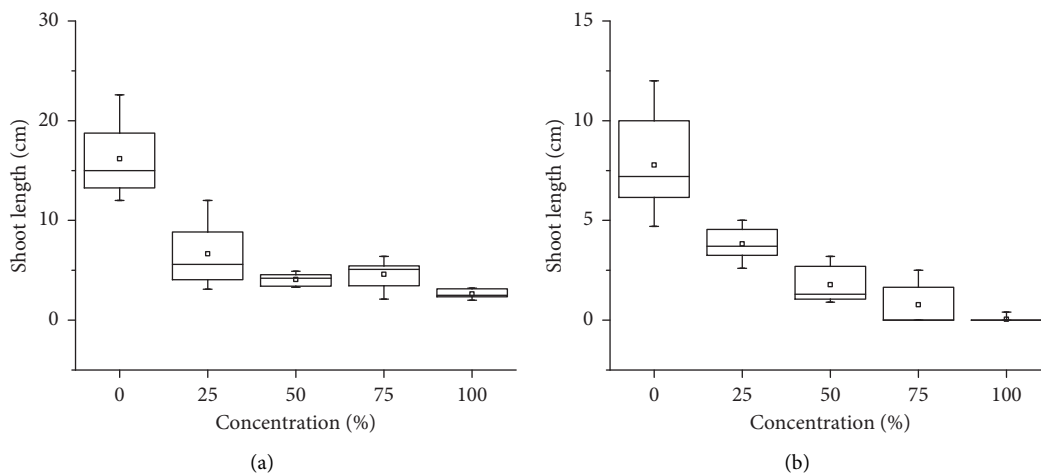


FIGURE 1: Shoot length of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in laboratory experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

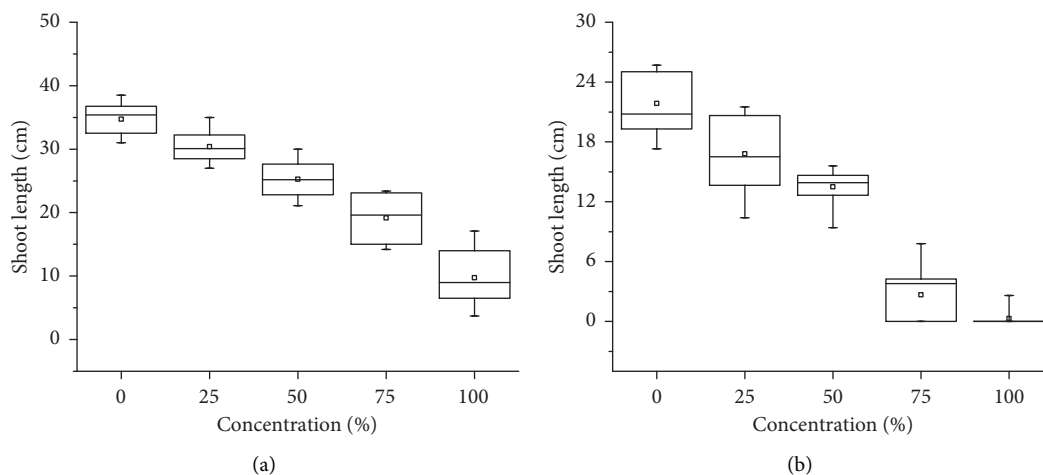


FIGURE 2: Shoot length of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in screen house experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

reduced seed germination in *P. vulgaris* and *O. sativa* might be caused by the allelopathic stress of different extract concentrations resulting from different abnormalities in metabolic activities and cell division due to the effect of allelochemicals [44]. This is reported to affect the

productivity of *P. vulgaris* and *O. sativa* in different farming systems, thereby lowering yields.

The findings in this study also indicate that root and shoot lengths of *P. vulgaris* and *O. sativa* were significantly reduced by the *S. suaveolens* crude extracts. However, the

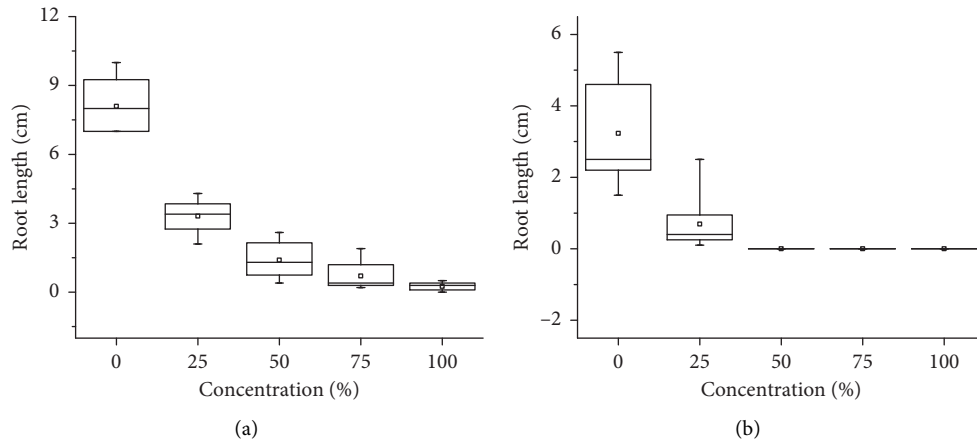


FIGURE 3: Root length of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in a laboratory experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

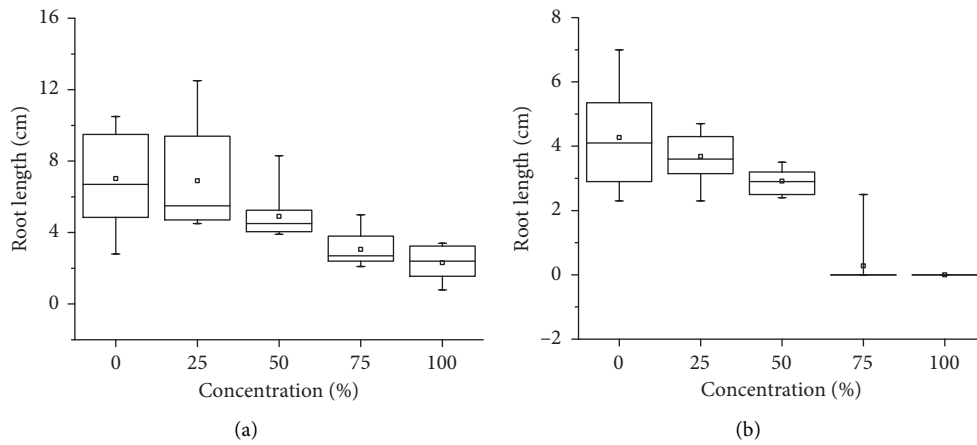


FIGURE 4: Root length of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in screen house experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

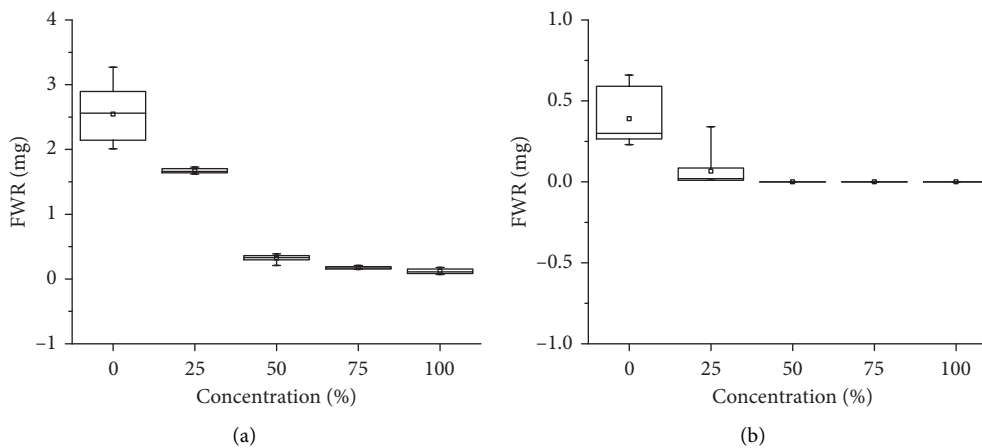


FIGURE 5: Fresh weight of root (FWR) of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in laboratory experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

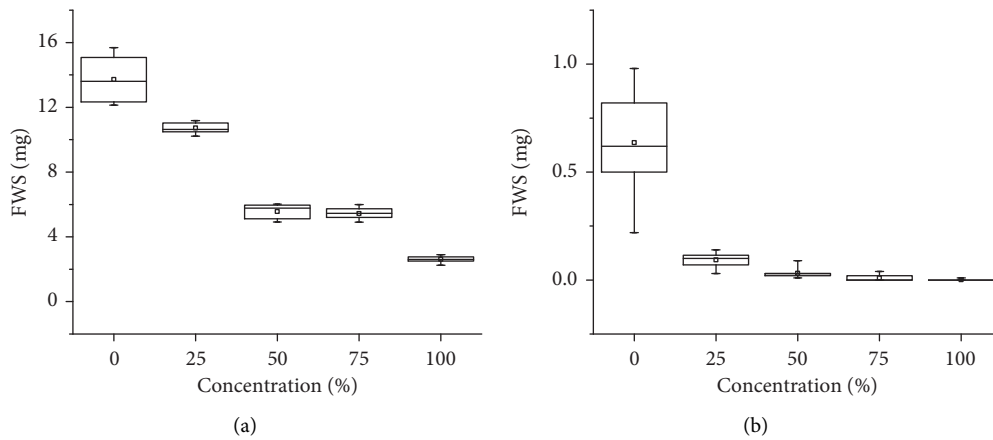


FIGURE 6: Fresh weight of shoot (FWS) of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in laboratory experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

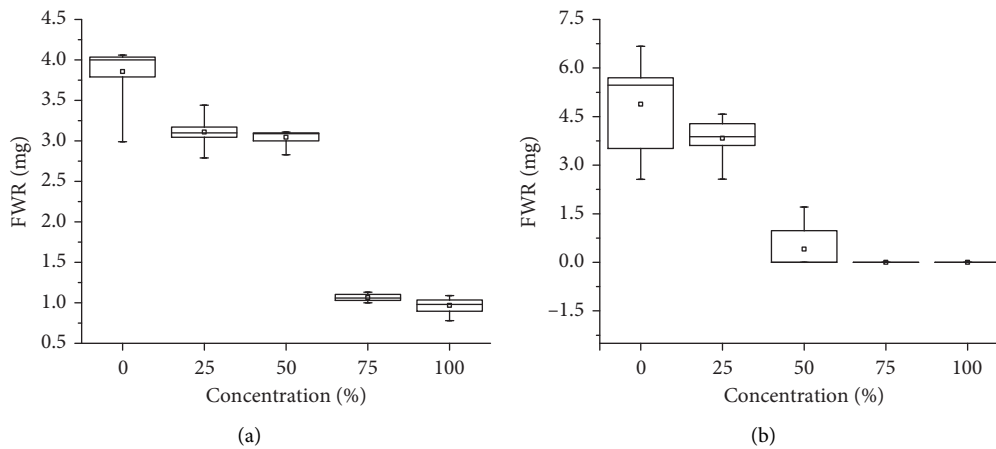


FIGURE 7: Fresh weight of root (FWR) of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in screen house experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

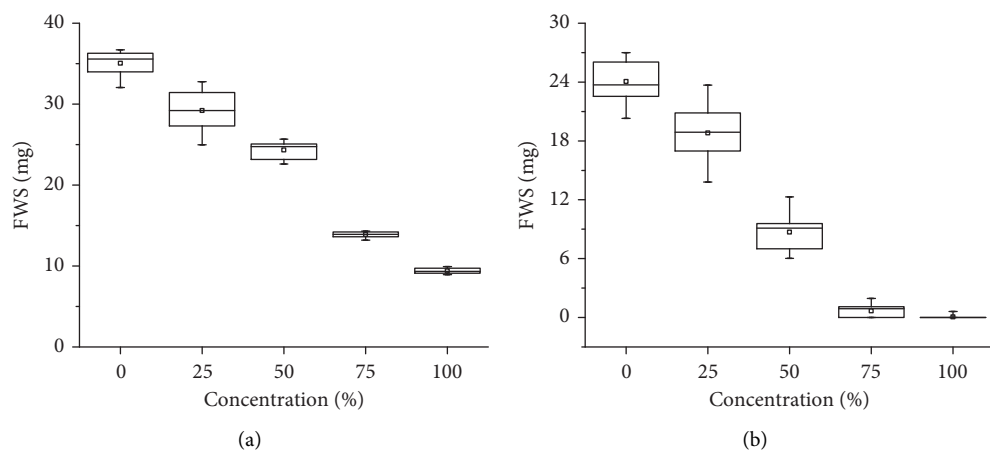


FIGURE 8: Fresh weight of shoot (FWS) of germinated *P. vulgaris* (a) and *O. sativa* (b) seedlings in screen house experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

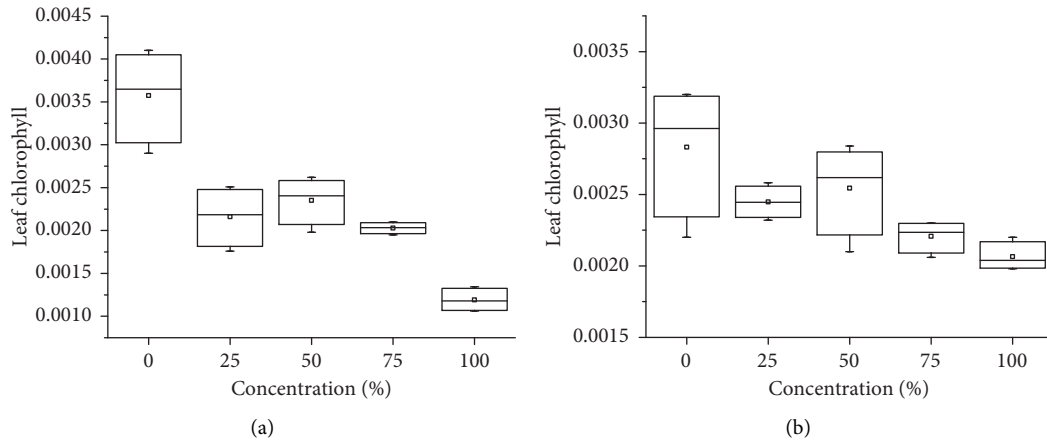


FIGURE 9: Leaf chlorophyll content of *P. vulgaris* (a) and *O. sativa* (b) seedlings in laboratory experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

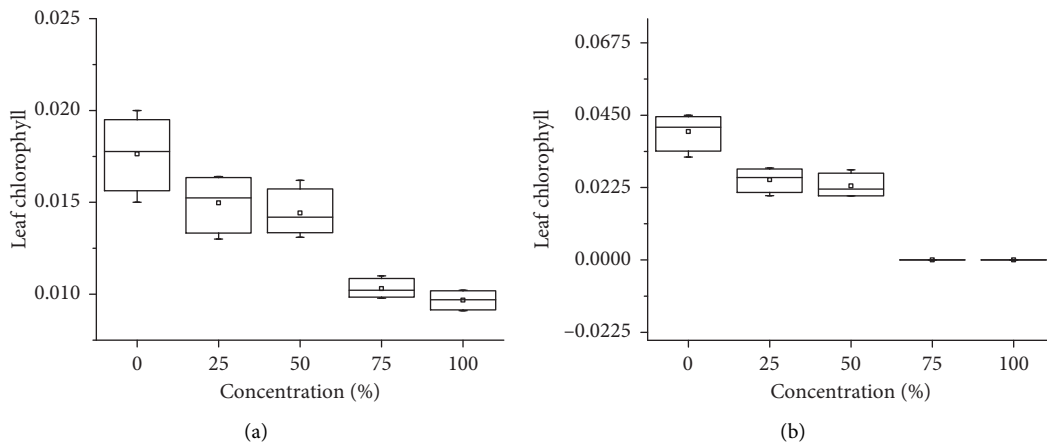


FIGURE 10: Leaf chlorophyll content of *P. vulgaris* (a) and *O. sativa* (b) seedlings in screen house experiment after 7 and 14 days of treatment with *S. suaveolens* extracts, respectively.

effects were concentration dependent and differed between the two tested crops (*P. vulgaris* and *O. sativa*). The roots and shoots of *O. sativa* were found to be more sensitive to the applied allelopathic stress than *P. vulgaris*, whereby at high concentration (100%) of crude *S. suaveolens* extract, root and shoot length was reduced considerably as compared with those of *P. vulgaris* at lower concentrations and in control treatments. These results corroborate with findings from Lodha [42] who revealed that extracts of different plant parts of *S. indicus* weed had strong inhibitory effects and reduced seed germination by 80% and root length and stem length by 94.4% and 83.3%, respectively, of *O. sativa*. Root and shoot lengths are very important parameters which determine plants' growth and health due to their importance in nutrients uptakes and physical support of the plant.

The reduced root and shoot lengths observed in this study may in one way or the other negatively affect crop production particularly in smallholder farming systems. The association between shorter roots and failure of plants to compete and search for water and minerals from the ground

has been well reported by Sofi et al. [45], Subudhi et al. [46], and Yamane et al. [48]. On the other hand, shorter shoots have been associated with plants' inability to withstand environmental stresses such as drought [48]. Also, shorter shoots hinder plants' ability to compete for space, light, and air which are important parameters during photosynthesis and their shortage may result into poor plant growth [49]. Additionally, Laizer et al. [7] and Lodha [42] reported lower *P. vulgaris* and *O. sativa* yields, respectively, in farms that were invaded with *S. suaveolens*. The low yields may have been attributed to the allelopathic effects of *S. suaveolens* which negatively affect root and shoot lengths.

Furthermore, results from this study show that, fresh weights of shoots and roots for *P. vulgaris* and *O. sativa* were significantly affected by the higher concentrations of *S. suaveolens* in both laboratory and screen house experiments. The seedling fresh weight is an important factor for a plant to withstand physical stresses from the environment [49]. Therefore, affecting the fresh weight of the *P. vulgaris* and *O. sativa* may affect their ability to withstand harsh

environmental conditions. The chlorophyll content of both *P. vulgaris* and *O. sativa* was also negatively affected by the *S. suaveolens* crude extract. The lower chlorophyll content observed in this study was due to the presence of allelochemicals found in the *S. suaveolens*. These findings were also reported by Frabboni et al. [50], Ngondya et al. (41), Ojija et al. (51), Rawat et al. [52], and Siyar et al. [44]. Reduced chlorophyll content may negatively affect plant's ability to perform photosynthetic functions, hence lowers the chance to survive or compete with other neighboring plants [53].

5. Conclusion

The findings from this study are among the first to demonstrate effects of *S. suaveolens* crude extracts to seed germination and growth of *P. vulgaris* and *O. sativa*. The results further show that *O. sativa* is more sensitive to the applied allelopathic stress than *P. vulgaris*. This might be due to its genomic characteristics which influence tolerance levels to chemical and other environmental stresses. At higher concentrations (75% and 100%), the *S. suaveolens* crude extract exerted deleterious effect on seed germination and seedling growth for both *P. vulgaris* and *O. sativa* compared with lower concentrations (25%) and the control (0%). These effects might be caused by the presence of the water-soluble allelochemicals in *S. suaveolens* crude extracts which are largely unknown and need to be isolated, identified, and characterized for profound understanding and further investigations on their applications in agriculture and other fields.

Data Availability

The germination and seedling growth data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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