

PLANT SCIENCE

Plant growth inhibitory activity of medicinal plant *Hyptis suaveolens*: could allelopathy be a cause?

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Abstract

The present study was conducted to explore the allelopathy of *Hyptis suaveolens* Poit, an important medicinal plant of Lamiaceae family. The aqueous methanol extracts of this plant at four different concentrations (3, 10, 30 and 100 mg dry weight [DW] equivalent extract/mL), were examined on the seedling growth of eight test plant species, cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus* L.), timothy (*Phleum pratense* L.), crabgrass (*Digitaria sanguinalis* L. scop.), barnyardgrass (*Echinochloa crus-galli* L.) and Italian ryegrass (*Lolium multiflorum* Lam.), and on the germination of cress and Italian ryegrass. The germination of cress, Italian ryegrass and the growth of all eight test species were significantly inhibited by the *H. suaveolens* plant extracts at a concentration greater than 3, 30 and 10 mg DW equivalent extract/mL, respectively. The inhibitory activity of the extracts was concentration dependent. The root growth of all the test plants was more sensitive to the extracts than the hypocotyl/coleoptile growth of those. The concentrations required for 50% inhibition (defined as I_{50}) of the hypocotyl/coleoptile and root growth of the eight test plant species range from 9.3–79.3 and 4.9–29.5 mg DW equivalent extract/mL, respectively. The hypocotyl growth of lettuce and the root growth of crabgrass were most sensitive to the extract, whereas coleoptile growth of barnyardgrass and the root growth of alfalfa were the least sensitive. The inhibitory activities of the *H. suaveolens* on the germination and growth of the test plant species suggest that the plant has allelopathic potentiality and may possess allelochemicals. These allelochemicals might be responsible for the restricted growth of other plant species near their colony in natural ecosystems. However, isolation and identification of these allelochemicals from *H. suaveolens* plant extracts could serve as the lead for new natural herbicides development for sustainable weed management strategies.

Key words: Allelochemicals, Weed management, Sustainable agriculture, Lamiaceae, Natural herbicide

Introduction

The initial use of synthetic herbicides to control weeds in the crop fields superficially increased the crop production by reducing the weed infestation. Eventually the excessive use of synthetic herbicide in the crop fields may obviously lead to a tremendous environmental hazards resulting in degradation of agricultural land by abolishing soil-biota (Pell et al., 1998); ground water contamination (Aktar et al., 2009); reduction of fisheries (Khan and Law, 2005); development of herbicide resistant weeds (Vyvyan,

2002); destruction of beneficial predators of pests and thereby increased the virulence of many species of agricultural pests (Wilson and Tisdell, 2001). To avoid the hurtful effects of synthetic herbicide, research on novel natural plant products have moved from the fringe to the mainstream for the development of ecologically acceptable, environment friendly, cost-effective and relatively safe natural herbicides.

Allelopathy of medicinal plants could play a vital role in identification of new allelochemicals and could accelerate the process of new natural herbicides development. Currently, many researches around the world show their keen interest on medicinal plants for searching new novel compounds and reported that medicinal plants have growth inhibitory effects on different noxious weed species and have the potentiality to use them in the crop fields either directly or as a natural herbicides (Lin et al., 2003, 2004; Han et

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al., 2008; Sodaeizadeh et al., 2009; Li et al., 2009). Moreover, it was reported that screening of allelopathic plant from medicinal plants species is easier than other plants (Fujii et al., 2003) possibly due to their existed certain metabolic compounds which was used for curing many diseases of both animal and human being. On the other hand, there are about 400,000 secondary metabolites in plants with allelopathic activities (Swain, 1977), of which only a few have been examined (Einhellung and Leather, 1988). The rest of the compounds, might contain very promising growth inhibitors are still unknown. Since about 12.5% of the total plants species of the world are considered as medicinal plants (Wakdikar, 2004), therefore, they could be served as important candidates for allelopathic research. Isolation and characterization of that unknown allelochemicals from medicinal plants might provide the chemical basis for new natural herbicides developments.

Hyptis, the genus of Lamiaceae contains approximately 400 species (Willis, 1973) and most of the species are native to the tropical America (Hutchinson and Dalziel, 1963; Hickey and King, 1988). However, one of the species *Hyptis suaveolens* Poit distributed throughout the tropics and subtropics, along the rail tracks, roadsides (Mishra and Verma, 1992), foothills of open forests, forest clearings (Mudgal et al., 1997) and also in the fallow lands. *H. suaveolens* has many medicinal properties and used for several ethnobotanical applications (Adda et al., 2011). For example, leaf decoction is used for the treatment of diabetes (Abdullahi et al., 2003) and cancer (Danmalam et al., 2009), and root decoction is highly valued as appetizer. Moreover, these organs contain urosolic acid, a natural HIV-integrase inhibitor (Chatterjee and Pakrashi, 1997). In addition, the leaves and twigs are considered as antispasmodic, antirheumatic, anti-inflammatory, antifertility agents (Kirtikar and Basu, 1991), and also have antiseptic, mosquitocidal and insecticidal properties (Shenoy et al., 2009; Adda et al., 2011). The leaves of *H. suaveolens* contain alkaloids, terpenes and volatile oils (Gills, 1992). Beside the pharmacological and toxicological properties of *H. suaveolens*, still now very few are known about its allelopathic activities. Therefore, the current research was conducted to explore the allelopathic activity of *H. suaveolens* on different test plant species.

Materials and Methods

Plant materials

Whole plants (leaves, stem and roots) of *H. suaveolens* were collected from Bangladesh during

the month of March-April, 2012. After collection, plants were washed with tap water to remove the soil and other debris followed by sun drying. The dried plants were then kept in a refrigerator at 2 °C temperature until extraction.

Test plant species

Eight test plant species, cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), rapeseed (*Brassica napus* L.), timothy (*Phleum pratense* L.), crabgrass (*Digitaria sanguinalis* L. scop.), barnyardgrass (*Echinochloa crus-galli* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) were selected for the present research. Among these eight test species, the first four are dicotyledonous and the rest are monocotyledonous. Cress, alfalfa, lettuce, rapeseed and timothy were chosen due to their known seedling growth, whereas crabgrass, barnyard grass and Italian ryegrass were chosen because they are most common weeds in the crop fields and distributed throughout the world.

Extraction procedure

The whole parts (leaves, stem and roots) of dried *H. suaveolens* (30 g) were cut into small pieces and extracted with 300 mL of 70 % (v/v) aqueous methanol for 48 h. After filtration using one layer of filter paper (No. 2; Advantec® Toyo Roshi Kaisha, Ltd., Tokyo, Japan), the residue was re-extracted with 300 mL of 100% methanol for 24 h and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C.

Germination bioassay

An aliquot of the extract (final assay concentration was 3, 10, 30 and 100 mg DW equivalent extract/mL) was evaporated to dryness at 40°C in *vacuo* by rotary evaporator, dissolved in methanol and added to a sheet of filter paper (No. 2) in a 2.8 cm Petri dish. The methanol was evaporated in a draft chamber then the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 (polyoxyethylene sorbitan monolaurate; Nacalai Tesque, Inc., Kyoto, Japan) which was used for surfactant and did not cause any toxic effects. Ten seeds of cress or Italian ryegrass were arranged on the filter paper in Petri-dishes. Control seeds were also sown on the filter paper moistened with Tween 20 without plant extracts. Then the Petri dishes were incubated in the dark chamber at 25°C. The germination bioassay was laid out using a completely randomized design with three replications. Seeds that showed the emergence of the radical by rupturing the seed coat were considered to be germinated as described by

Barrôco et al. (2005) and Faria et al. (2005). Germination of seeds was recorded at every 12 h intervals for four days. The percentage of germination over control in each treatment was calculated using the following equation:

$$\text{Germination (\% of control)} = \frac{G_T}{G_0} \times 100$$

Where,

G_T = average number of germinated seed in the treatment in each time of measurements

G_0 = average number of germinated seed in the control at the same time of measurements

Growth bioassay

Test samples of plant extracts were prepared and added to a sheet of filter paper (No. 2) in a 2.8 cm Petri dish, and the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 as described above. Then, 10 seeds of cress, lettuce, alfalfa, rapeseed or 10 germinated seeds of timothy (germinated in the darkness at 25°C for 72 h after overnight soaking), crabgrass (germinated in the darkness at 25°C for 120 h after 24 h incubation in the light chamber at 25°C), barnyardgrass or Italian ryegrass (germinated in the darkness at 25°C for 24 h after overnight soaking) were arranged on the filter paper in Petri-dishes. The hypocotyl/coleoptile and root lengths of the seedlings were measured at 48 h after incubation in darkness at 25°C. Control seeds were sown on the filter paper moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween 20 without plant extracts. The same bioassay experiment was repeated twice with three replications in each case.

The inhibition percentage was calculated using the following equation:

$$\text{Inhibition (\%)} = \left[1 - \frac{\text{length with aqueous methanol extract}}{\text{length of control}} \right] \times 100$$

The concentrations required for 50% inhibition (express as I_{50}) of the test plant species in the assay was calculated from the regression equation of the concentration response curves.

Statistical analysis

Experimental data were analyzed using statistical software PASW statistics 17.0 (SPSS Inc., Illinois, USA) and GraphPad Prism 6.0 (GraphPad Software, Inc., California, USA).

Results

Effects of *H. suaveolens* plant extracts on the germination of two test plant species

The effects of aqueous methanol extracts of *H. suaveolens* on the germination of cress and Italian ryegrass were determined (Figure 1, 2). The extracts inhibited the germination and the inhibition was concentration dependent. The two-way ANOVA indicates that the four different concentrations, the period of incubations and their interaction have significant effect ($p < 0.001$) on the germination of both cress and Italian ryegrass. The germination of cress was significantly inhibited by the *H. suaveolens* plant extracts at all extract concentrations. In contrast, the germination of Italian ryegrass was significantly inhibited at a concentrations ≥ 30 mg DW equivalent extract/mL (Figure 1, 2).

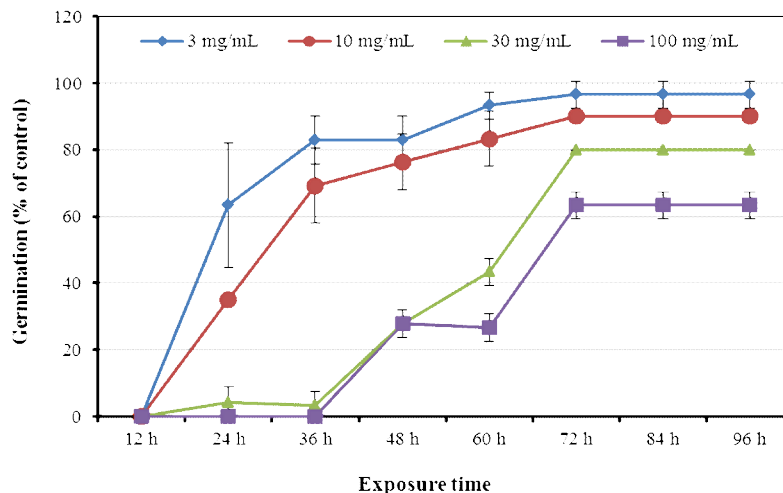


Figure 1. Effects of aqueous methanol extracts of *H. suaveolens* on the germination of cress. Means \pm SE from three independent experiments with 10 seeds for each determination are shown. All the values are statistically significant at $p < 0.001$.

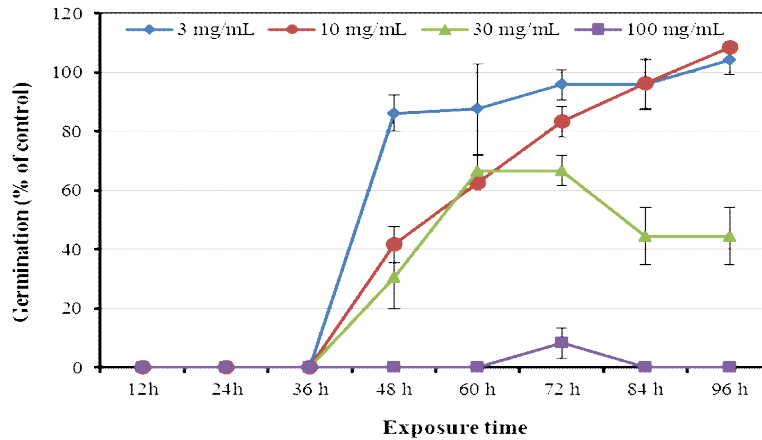


Figure 2. Effects of aqueous methanol extracts of *H. suaveolens* on the germination of Italian ryegrass. Means±SE from three independent experiments with 10 seeds for each determination are shown. All the values are statistically significant at $p < 0.001$.

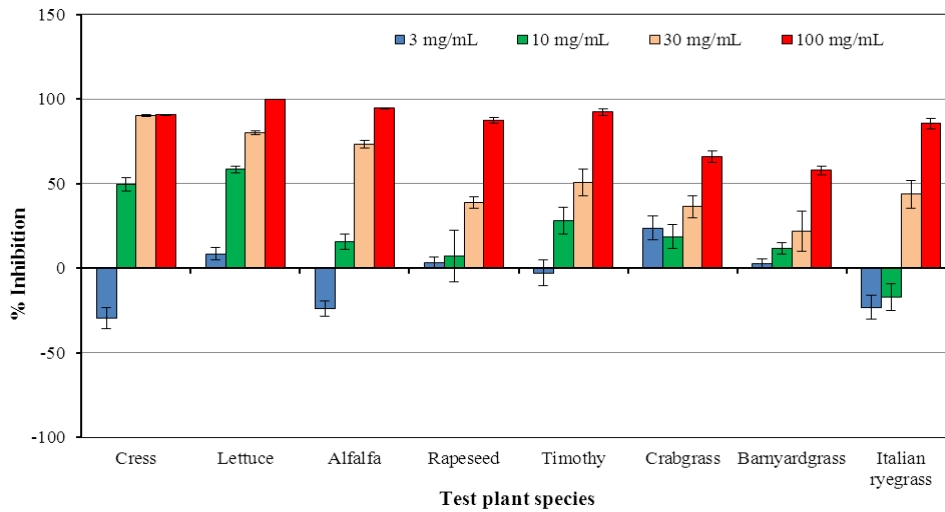


Figure 3. Effects of aqueous methanol extracts of *H. suaveolens* on hypocotyl/coleoptile growth of the test plant species.. Means±SE from three independent experiments with 10 seedlings for each determination are shown. The negative (-) value in the Y axis indicates stimulation and positive (+) value indicates inhibition of the hypocotyl/coleoptile growth of eight test plant species by *H. suaveolens* plant extracts.

Effects of *H. suaveolens* plant extracts on the growth of eight test plant species

The inhibition percent of the aqueous methanol extracts of *H. suaveolens* on the hypocotyl/coleoptile and root growth of cress, lettuce, alfalfa, rapeseed, timothy, crabgrass, barnyardgrass and Italian ryegrass are shown in Figures 3 and 4. The hypocotyl/coleoptile and root growth of all but Italian ryegrass and alfalfa were significantly inhibited by the

extracts at concentrations greater than 3 mg DW equivalent extract/mL. Moreover, the effectiveness of *H. suaveolens* plant extracts were different among test species, and the inhibition percent of the extracts was concentration dependent (Figure 3, 4). The two-way ANOVA showed that *H. suaveolens* plant extracts and its interaction with the eight test plant species has a significant ($p < 0.001$) effect on the seedling growth of all the test plant species.

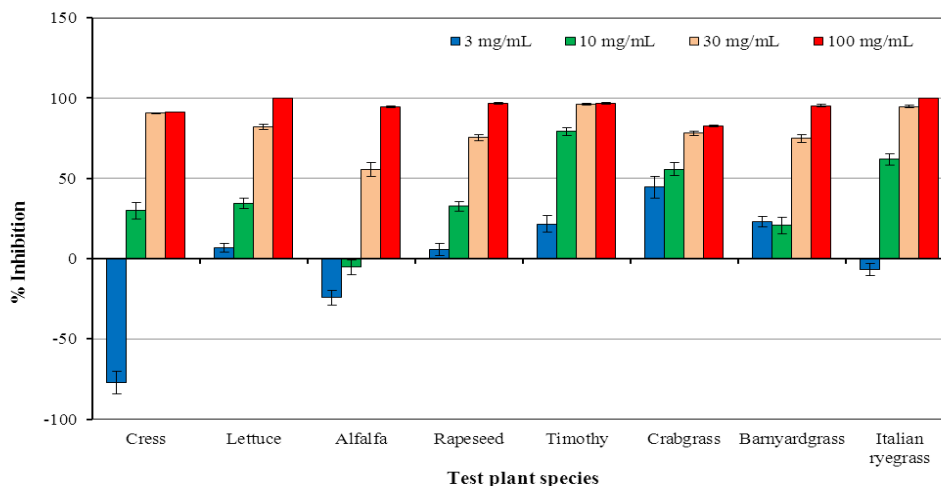


Figure 4. Effects of aqueous methanol extracts of *H. suaveolens* on root growth of the test plant species. Means±SE from three independent experiments with 10 seedlings for each determination are shown. The negative (–) value in the Y axis indicates stimulation and positive (+) value indicates inhibition of the root growth of eight test plant species by *H. suaveolens* plant extracts.

At the concentration of 30 mg DW equivalent extract/mL, the inhibition percent of the aqueous methanol extracts of *H. suaveolens* on hypocotyl/coleoptile growth of cress, lettuce, alfalfa, rapeseed, timothy, crabgrass, barnyardgrass and Italian ryegrass was 90, 80, 73, 39, 51, 36, 22 and 44, respectively (Figure 3). The hypocotyl growth of lettuce seedling was completely inhibited (Figure 5) when the seeds are applied to a concentration of 100 mg DW equivalent extract/mL, followed by alfalfa, timothy, cress, rapeseed, Italian ryegrass, crabgrass and barnyardgrass at 95, 92, 91, 87, 86, 66 and 58% inhibition, respectively. On the other hand, the extracts of *H. suaveolens* stimulated the hypocotyl/coleoptile growth of cress, alfalfa, timothy and Italian ryegrass at a concentration of 3 mg DW equivalent extract/mL. In addition, the coleoptile growth of Italian ryegrass was stimulated by the *H. suaveolens* at a concentration of 10 mg DW equivalent extract/mL (Figure 3). Considering the concentration required for 50% inhibition (defined as I_{50}), it was revealed that the hypocotyl growth of lettuce seedling was most sensitive to the aqueous methanol extracts of *H. suaveolens*, whereas the coleoptile growth of barnyardgrass was least sensitive (Table 1).

At the concentration of 30 mg DW equivalent/mL, the inhibition percent of the root growth of timothy, Italian ryegrass, cress, lettuce, crabgrass, rapeseed, barnyardgrass and alfalfa was 96, 95, 91, 82, 78, 75, 75 and 56, respectively (Figure 4). When the test plant species were

exposed to the concentration of 100 mg DW equivalent extract/mL, the root growth of lettuce and Italian ryegrass seedling were completely (100%) inhibited (Figure 5, 6) followed by rapeseed, timothy, barnyardgrass, alfalfa, cress and crabgrass seedlings and the inhibition on their root growth was 97, 97, 95, 95, 91 and 83%, respectively. In contrast, the root growth of cress, alfalfa and Italian ryegrass were significantly stimulated by the *H. suaveolens* plant extracts at the concentration of 3 mg dry weight equivalent extract/mL (Figure 4). Furthermore, the root growth of alfalfa was stimulated by the extracts at a concentration of 10 mg DW equivalent extract/mL (Figure 4). Considering I_{50} value, the root growth of crabgrass was most sensitive to the *H. suaveolens* plant extracts than the other test plant species, whereas alfalfa was least sensitive to the extracts (Table 1).

Discussion

H. suaveolens is a ruderal type of plants that normally grows in a colony. It was reported that the other plant species near their colony is quite restricted (Raizada, 2006), but there have not so much clear evidence of the reasons. However, one of the main reasons for this character of the plant could be due to its allelopathic potentiality. A few evidences are found in the literature about the allelopathic activity of the leaf extracts and dry leaf residue of *H. suaveolens* plants. For example, Chatiyanon et al. (2012) reported that the water and methanol extract of the leaves of *H. suaveolens* has

allelopathic effects on the germination and seedling growth of *Pennisetum setosum* (Swartz.) L.C. Rich and *Mimosa invisa* Mart. Similar findings were also reported by Kapoor (2011) who worked with dry leaf residue of *H. suaveolens* and observed inhibitory activity on the growth and physiological parameters of *Parthenium hysterophorus* L. In the present research the aqueous methanol extracts of *H. suaveolens* significantly inhibited the germination of cress and Italian ryegrass at concentrations greater than 3 and 30 mg DW equivalent extract/mL, respectively. The extracts also significantly inhibited the seedling growth of all test plant species (cress, lettuce, alfalfa, rapeseed, timothy, crabgrass, barnyardgrass and Italian ryegrass) at concentrations greater than 10 mg DW equivalent extract/mL. These results imply that the aqueous methanol extract of *H. suaveolens* may possess allelochemicals which are responsible for their inhibitory activity. Moreover, the inhibitory activity of *H. suaveolens* plant extracts on different test species was concentration dependent. Concentration dependent inhibition on germination and growth by allelopathic plants extracts was also reported by Inderjit and Keating (1999), Kato-Noguchi et al. (2001), An et al. (2005), Bogatek et al. (2006) and Soltys et al. (2012).

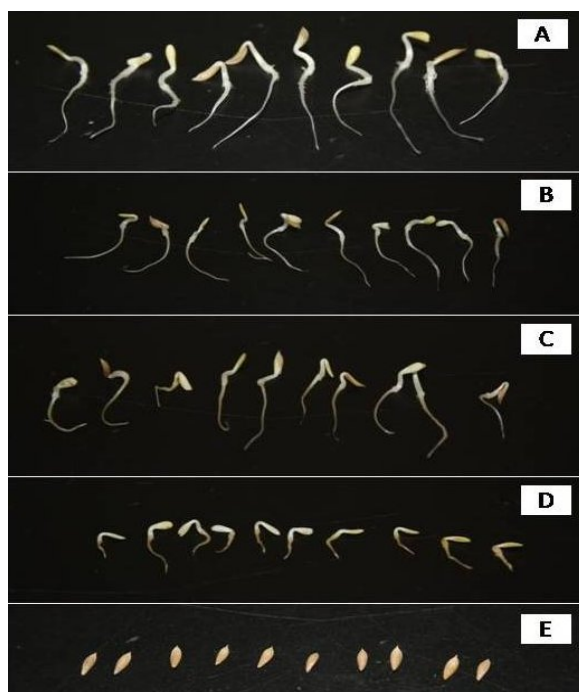


Figure 5. Effects of aqueous methanol extracts of *H. suaveolens* on the seedling growth of lettuce, after 48h incubation in the darkness at different concentration. Here, A, B, C, D and E denotes the concentration of 0, 3, 10, 30 and 100 mg dry weight equivalent extract/mL, respectively.

Table 1. I_{50} values of the aqueous methanol extract of *H. suaveolens* plant on hypocotyl/coleoptile and root growth of eight test plant species.

Test plant species	I_{50} (mg dry weight equivalent extract/mL)	
	Hypocotyl/Coleoptile growth	Root growth
Cress	10.0	11.7
Lettuce	9.3	13.8
Alfalfa	20.3	29.5
Rapeseed	37.7	15.4
Italian ryegrass	30.6	9.5
Barnyardgrass	79.3	13.2
Crabgrass	51.6	4.9
Timothy	25.3	5.4

Note: The values were determine by a logistic regression analysis after bioassays.

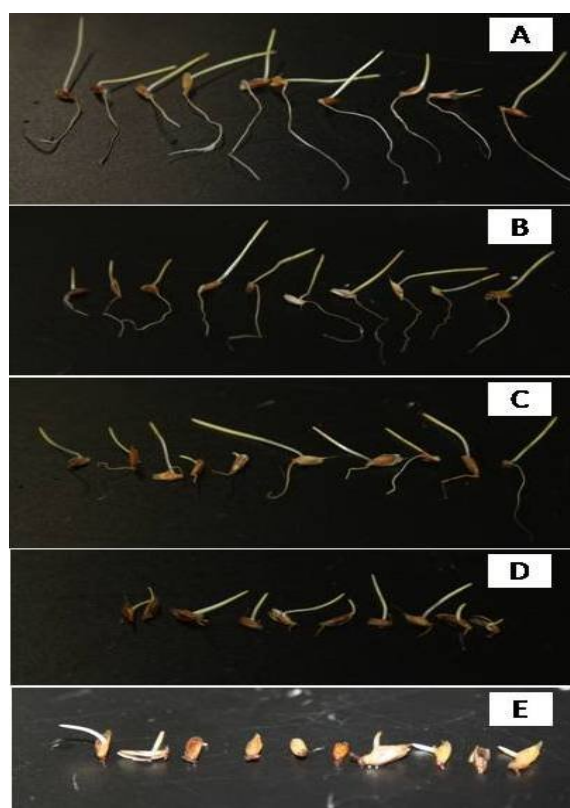


Figure 6. Effects of aqueous methanol extracts of *H. suaveolens* on the seedling growth of Italian ryegrass, after 48 h incubation in the darkness at different concentration.

Here, A, B, C, D and E denotes the concentration of 0, 3, 10, 30 and 100 mg dry weight equivalent extract/mL, respectively.

The inhibitory activity of *H. suaveolens* plant extracts on the germination of cress and Italian ryegrass was congruent with the previous findings of many other researchers. They reported that the decrease of germination ability in presence of allelochemicals could be due to several

abnormalities created by the allelochemicals on seed during germination process. For example, Kato-Noguchi and Macías (2006) reported that allelochemicals like 6-methoxy-benzoxazolin-2(3H)-one (MBOA) inhibit the germination of cress seeds by inhibiting the induction of α -amylase activity, which is very crucial for the conversion of reserve carbohydrate into soluble sugars during seed germination. Oracz et al. (2007) stated that the accumulation of reactive oxygen species caused cellular damage, which resulted in the decrease of germination ability and gradual loss of seed vigour. The decrease in germination ability was also due to enhanced membrane deterioration (Bogatek et al., 2006).

The root growth of all the test plant species was observed to be more sensitive to the *H. suaveolens* plant extracts than the hypocotyl/coleoptile growth. These results are in agreement with the earlier findings of Stachon and Zimdahl (1980) and Aliotta et al. (1993) who reported that allelopathic plant extracts had higher root growth inhibition than the coleoptiles. This phenomenon might be due to the more intensive contact between roots and plant extracts. Salam and Kato-Noguchi (2010) also reported that roots were more sensitive to the allelochemicals than hypocotyls/coleoptiles because the roots are the first organ to absorb allelochemicals from the environment. Whereas, Nishida et al. (2005) stated that the permeability of allelochemicals into root tissue is higher than the shoot tissue. They also explained that the hypocotyl/coleoptile growth of seedlings largely depends on cell expansion which is relatively insensitive to the allelochemicals, whereas root growth requires not only cell expansion, but also cell proliferation which is sensitive to the allelochemicals and therefore, the root growth exerts higher inhibition than the hypocotyl/coleoptile growth.

On the other hand, a number of abnormalities have been found when the test species are subjected to allelochemicals, and is the plausible cause for their growth inhibition. For example, allelochemicals inhibit the process of cell division, elongation and expansion rate, (Rice, 1984; Ortega et al., 1988; Einhellig, 1996; Jacob and Sarada, 2012), respiration process (Inderjit and Keating, 1999), ion absorption process (Qasem and Hill, 1989), enzyme activity (Sato et al., 1982), plant endogenous hormones and protein synthesis (Jacob and Sarada, 2012), alteration of the phytochrome control of germination (Leather and Einhellig, 1988) and consequently, arrested the plant growth.

Allelochemicals may produce more than one effect of the above on the cellular processes that could be responsible for the reduced seedling growth of the test plant species. But the details of the biochemical mechanism through which allelochemicals exert a toxic effect on the growth of any plant species are still not well known (Zhou and Yu, 2006). Nevertheless, the stimulatory activity on the hypocotyl/coleoptiles and root growth of cress, alfalfa, timothy and Italian ryegrass by the aqueous methanol extract of *H. suaveolens* at a concentration less than 10 mg DW equivalent extract/mL are in line with the findings of many other researchers. They reported that allelochemicals can stimulate the seedlings growth at very low concentrations but inhibit the seedlings growth at high concentrations (Rice, 1984; Lovett et al., 1989; Liu and Chen, 2011; Islam and Kato-Noguchi, 2012). The stimulatory activity of any compound at low doses is called hormesis (Southam and Erlich, 1943). In some cases, allelochemicals have also been reported to induce hormesis. The reason behind the hormesis is due to the availability of some chemicals at lower doses which could affect the plant hormones that are responsible for shoot or root elongation, while they might have inhibitory activity on the seedling growth at higher doses due to the same or another mechanism of action (Duke et al., 2006).

Conclusion

The concentration dependent inhibitory activities of the aqueous methanol extracts of *H. suaveolens* on the germination and seedling growth of the test species suggest that the plant has allelopathic potentiality and possess allelochemicals. These allelochemicals could be the main reason for the restricted growth of other plant species near their colony. Isolation and identification of those allelochemicals from *H. suaveolens* could be act as lead for the development of bio-degradable, environment friendly new natural herbicides for sustainable weed management strategies. However, more research is necessary to further confirm the allelopathic potentiality of *H. suaveolens* under field and green house conditions to make our predictions more accurate.

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