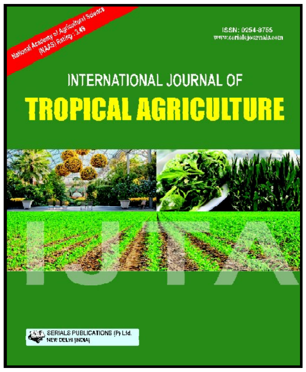

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Phytotoxic activity of essential oil of *Pogostemon benghalensis* against *Avena fatua* and *Parthenium hysterophorus*

Sangeeta Dahiya[1*] and Narayan Singh[1]

[1] Department of Botany, Panjab University, Chandigarh 160 014, India
** Corresponding Author: Sangeeta Dahiya*
Department of Botany, Panjab University, Chandigarh 160014, India E- mail: sangeetakvadav@gmail.com

Abstract: A study was conducted to explore the phytotoxic potential of *Pogostemon benghalensis* essential oil with an aim to determine its herbicidal activityl against two weeds *viz., Avena fatua* L. (wild oat) and *Parthenium hysterophorus* L. (congress grass). In laboratory bioassay, *Pogostemon* oil showed significant inhibitory effect on seed emergence and seedling length of both the test weeds. Oil treatment had more effect on seedling length of *P. hysterophorus* than *A. fatua*. Further, spray experiment was conducted under experimental dome conditions to evaluate phytotoxic activity of the oil. Under experimental dome conditions, *A. fatua* was found to be more sensitive than *P. hysterophorus*. When sprayed with *Pogostemon* oil emulsion, a declining pattern in seedling length and total chlorophyll content was recorded. The results from the present study suggested that *Pogostemon* oil possess phytotoxic potential and could thus serve as a strong candidate for weed management purposes.

Keywords: Essential oil, Percent germination, Phytotoxicity, Cellular respiration, Seed emergence, Chlorophyll content.

INTRODUCTION

Essential oils are concentrated lyophilic liquids containing volatile aromatic compounds that are synthesized within the plants as secondary metabolites. These play a vital role in plant defence mechanism against herbivores [1] and pathogens [2] as pollinator attractants [3] and also in plant-plant interactions [4], Essential oils have been extensively used in medicine, food, flavour and fragrance industry [5]. In addition, essential oils also possess antifungal, antimicrobial, nematicidal and insect repellent properties [6]. Several studies have reported that the phytotoxic effect of essential oils and their possible use as herbicides [6,7,8,9,10,11]. These studies are important because essential oils are environmentally safer, biodegradable, bioefficacious and possess novel sites of action [5,12,13].

Pogostemon benghalensis (Family Lamiaceae), an aromatic plant, grows wildly in the tropical regions and commonly occurs in open forests [14]. It is also cultivated in various countries like southern China, India, Sri Lanka, Nepal, Bangladesh, Myanmar and Thailand for its essential oil [15]. It is shrub having strong and angular stem. It bears pinkish-white bilipped flowers [16]. It produces strongly aromatic essential oil from its leaves, floral buds and flowers. Its essential oil shows wide spectrum of biological activities and have a great medicinal value. It is used as an aphrodisiac, antidepressant and antiseptic and also in aromatherapy to treat skin problems [17]. However, the role of essential oil in inhibiting seed germination and growth of test plants especially weeds remains largely unknown. We, therefore, extracted essential oil from the above ground parts of the plant and assessed its phytotoxicity against *P. hysterophorus,* an obnoxious weed of wasteland and *A. fatua* that interferes with the wheat growth.

MATERIALS AND METHODS

Collection of material

Pogostemon benghalensis grows abundantly in and around Chandigarh. Collection of plant material was done from late December to April, when the plant is in its flowering stage. The seeds of selected weeds (*P. hysterophorus* and *A. fatua*) were collected from the wildly growing stands and agricultural field in and around Chandigarh.

Extraction of oil

The essential oil was extracted from the above the ground parts in the flowering stage of *P. benghalensis* using Clevenger's apparatus through hydro-distillation and was referred to as *Pogostemon* oil. It was stored at 4°C for further use in bioassay.

Growth bioassay

Pogostemon oil was assessed for the phytotoxic activity ranging from 0.25 mg/ml – 2.5 mg/ml under laboratory condition. Seeds of *Avena fatua* were soaked in distilled water for 24 h while seeds of *Parthenium hysterophorus* were imbibed for 48 h. To determine the phytotoxic effect, solution of oil were prepared with the help of Tween 20 (a surfactant). Seeds of weeds were placed equidistantly placed in Petri dishes (15 cm) lined with a thin layer of cotton and Whatman #1 filter paper. Filter papers were treated with oil solution of different concentrations. Petri dishes were then sealed with brown tape. A similar set up but without essential oil served as control. For each concentration, five replicates were maintained. Entire set of *P. hysterophorus* were kept in growth chamber set at 25±2 °C while set of *A. fatua* were kept at 15±2 °C. After seven days, seedling length of germinated weeds was measured.

Spray experiment

Seeds of each weed were sown in 15 cm diameter polypropylene pots in a greenhouse. A mixture of soil, sand and farmyard manure was prepared (ratio is 3:1:1). Then pots were filled by this mixture of soil. Initially 10-15 seeds were sown, but later on these were thinned to 4-5 plants per pot. After 4 weeks, plants were spray treated with oil solution of different concentration (0, 0.5%, 1%, 2.5% and 5%) were prepared with the help of Tween 20. Five replicates of each concentration were maintained along with control. After 14 days of spraying, treated plants were uprooted and evaluated in term of seedling length, dry weight and for the determination of total chlorophyll content.

Chlorophyll content

The chlorophyll was extracted in DMSO (dimethyl sulphoxide) as suggested by Hiscox and Israelstam

[18] and was measured spectrophotometrically. The calculations were done as per Arnon's equation [19]. The final expression of the chlorophyll was done on the basis of dry weight as suggested by Rani and Kohli [20].

Statistical analysis

All the experiments were conducted in completely randomised block design and were repeated. Mean data of two experiments is represented. Dose response curve was derived by using GraphPad Prism version 6.0. Data were subjected to one way ANOVA and treatment were compared at $P \leq 0.05$. Later on, data were subjected to linear regression. All statistical analysis was performed using SPSS software version 16.0.

RESULTS

In the above experiments, it had been found that the essential oil has a significant effect on percent germination. The *Pogostemon* oil inhibited the germination in a dose dependent manner. The inhibitory effect of the oil increased with increasing concentration. Regarding the germination inhibition, seeds of weed *P. hysterophorus* were more sensitive compared to that of *A. fatua* because a complete germination inhibition of seed was observed at concentration 0.75 mg/ml, while, in *A. fatua* complete germination inhibition could be seen at 2.5 mg/ml concentration (Fig. 1a).

The essential oil not only inhibited germination but also had a negative impact on seedling growth. In laboratory bioassay, at lower concentration, effect was found to be less as compared to control ones, but effect increases with increase in concentration of oil emulsion. A significant (at $P \leq 0.05$) reduction in seedling length was observed at lower concentration (at ≥ 0.25 mg/ml) in the test weeds. Greater inhibition on seedling length was observed in case *P. hysterophorus* followed by *A. fatua*. At higher concentration, nearly 38-100% and 39-100%

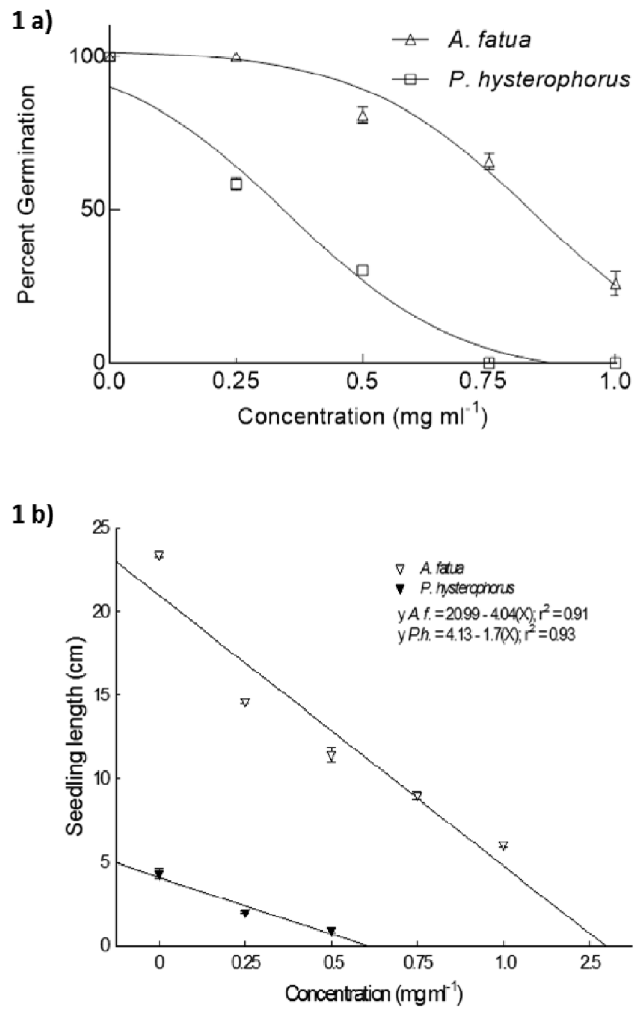

Figure 1: (a) Phytotoxic effect of Pogostemon oil the seed germination (expressed as % germination). Bar represent ± S.E. b) Regression lines indicating pre-emergent effect of *P. benghalensis* essential oil on seedling length of both weeds under laboratory condition. Data were analysed by linear regression using SPSS verion 16.0. Data represents mean ± Standard error. r^2 represents coefficient of determination. All regressions were significant at $P \leq 0.05$

reduction in seedling length was observed in *A. fatua* and *P. hysterophorus,* respectively (Fig. 1b). Under experimental dome condition, spray treated mature plants showed some symptoms of visible injury including discolouration of leaves, yellowing of leaves, etc. *A. fatua* showed more visible injury as compare to *P. hysterophorus.* At highest concentration 5%, *A. fatua* showed 60-80% injury level while *P. hysterophorus* showed 40-60% as compared to control (Table 1).

Table 1
Effect of *Pogostemon* oil (after 14 days of spray) on injury level of the test weeds. +: injury level in the range of 0-20%; ++: 20-40%; +++: 40-60%; ++++: 60-80%; +++++: 80-100%

Concentration (% v/v)	*A. fatua*	*P. hysterophorus*
0	0	0
0.5	+	0
1.0	++	+
2.5	+++	++
5.0	++++	++

Further, significant ($P \leq 0.05$) reduction in plant height was observed at lower concentration (0.5%) in *P. hysterophorus* while *A. fatua* showed significant reduction at ≥ 1.0%. At highest concentration, nearly 7-53% and 13-51% reduction in plant height was observed in case of *A. fatua* and *P. hysterphorus,* respectively (Fig. 2a). The reduction was more in *A. fatua* than *P. hysterphorus.* Not only plant height, total chlorophyll content of selected weeds reduced in response to the treatment of essential oil. Chlorophyll content was found to be significantly ($P \leq 0.05$) reduced at lower concentration (1 % v/v) in both the test weed.

At highest concentration, nearly 20-93% and 39-75% reduction in total chlorophyll content were observed in case of *A. fatua* and *P. hysterophorus,* respectively (Fig 2b). Among both the test weeds, highest reduction in chlorophyll content was observed in case of *A. fatua* followed by *P. hysterophorus*. Similarly, a significant effect of essential oil on dry weight of weed plant was reported. All the selected test weeds showed significant reduction at concentration ≥0.5 mg/ml. On treatment, *A. fatua* and *P. hysterophorus* showed nearly 15-70% and 20-

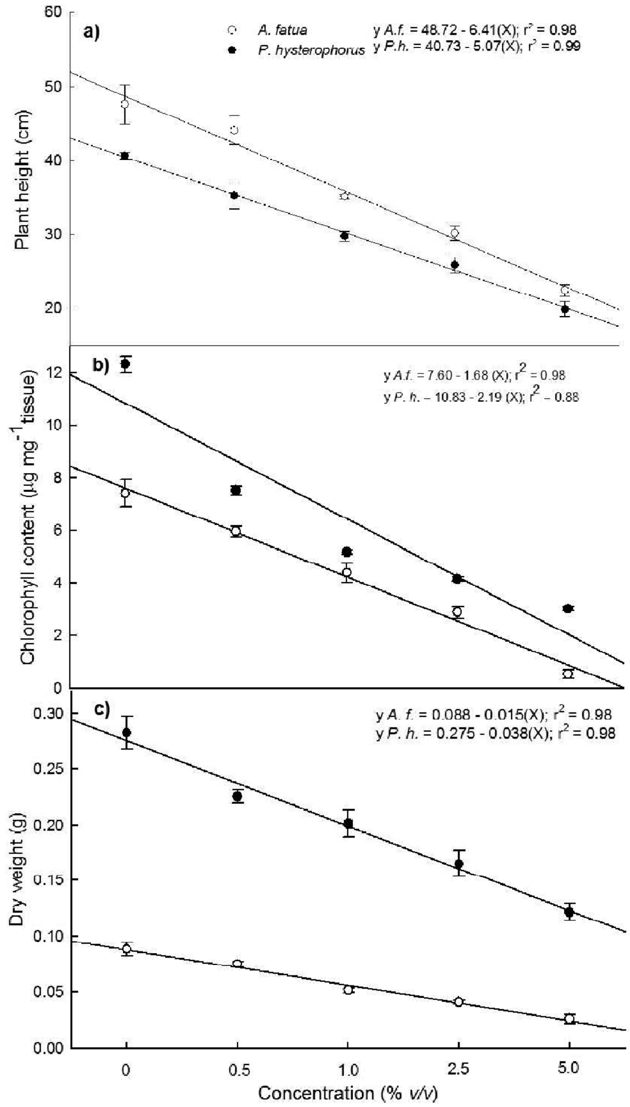

Figure 2: Regression lines indicating post-emergent effect of *P. benghalensis* essential oil on Plant height (a) Chlorophyll content (b) and Dry weight (c) of weeds under experimental dome condition. Data were analysed by linear regression using SPSS verion 16.0. Data represents mean ± Standard error. r^2 represents coefficient of Determination. All regressions were significant at $p \leq 0.05$

57% significant reduction in dry weight, respectively with respect to control as shown in Fig 2c.

DISCUSSION

Owing to their novel mode of actions, essential oils and their constituents are ecologically acceptable pesticides. *Pogostemon* essential oil is a complex mixture of monoterpenes, oxygenated and hydrocarbon sesquiterpenes. Earlier, Azulen-2-ol, 1,4-dimethyl-7-(1-methylethyl)- was reported as a major compound (peak area 41.72%) which is an oxygenated sesquiterpene [14]. As per the result of previous study, *Pogostemon* oil is rich in α-Gurjunene, -Cadinol, Cadina-1 (10), 4-diene, Humulene, Germacrene D, α- Ocimene, Carotol, Caryophyllene etc [21]. From the earlier studies, it could be concluded that *Pogotsemon* oil is rich in sesquiterpenes followed by monoterpenes [14, 21, 22, 23]. There are several research reports claiming phytotoxic nature of sesquiterpens [24, 25, 26, 27] and monoterpens [28, 29, 30]. Generally, both monoterpenes and sesquiterpenes, synergistically play a crucial role to the phytotoxicity. In the present study, we found that the essential oil has an inhibitory effect on seed the emergence. These observations are parallel to earlier studies documenting to the phytotoxic nature of essential oil to other plant [12, 31, 32, 33, 34, 35, 36]. The present study clearly depicts that essential oil of *P. benghalensis* is phytotoxic as evident from its inhibitory effects. Some earlier studies have reported that the volatile monoterpenes are potent inhibitors of mitosis [8, 30, 32, 36, 37]. Thus, inhibition/distruption in mitotic activity may be one of the possible reasons for the retardation in the seedling growth. However, the exact mechanism of inhibition of seed emergence is still unknown. Though, it has been reported that the essential oil causes various morphological and physiological alteration in the test plant [12, 31, 32].

It has been documented that essential oils and their constituents (monoterpenes and sesquiterpenes) cause damage to cells by generating the reactive oxygen species which further causes damage to cells by lipid peroxidation and membrane distintegration. Generation of ROS in excess may also be one of the possible reasons of growth retardation [38, 39].

In the present investigation, a decline in the total chlorophyll content has been observed in plants spray treated with oil. Yellowing and discoloration of leaves might be a result of loss of chlorophyll after spraying oil emulsion. Although the exact mechanism of decrease in the total chlorophyll content due to essential oil is unknown, it could be due to either inhibition of chlorophyll synthesis or due to enhanced degradation of chlorophyll [8,40,41]. In both the cases, it is going to affect chlorophyll content negatively and retard overall growth of plant.

CONCLUSION

From the present study, it can be concluded that essential oil extracted from aerial parts (leaves and floral buds) of *P. benghalensis* exhibits strong phytotoxicity towards the test weed and hence could be useful for developing herbicides based on natural plant product. However, these studies are preliminary and require further investigations.

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