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Phytotoxicity of *Callistemon viminalis* essential oil against some weeds

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Abstract: The present study investigated the phytotoxic potential of *Callistemon viminalis* essential oil against some weeds *viz. Ageratum conyzoides*, *Sorghum halepense*, *Leptochloa chinensis* and *Commelina benghalensis* in order to assess its herbicidal activity. The laboratory bioassay revealed that *Callistemon* oil (0.025-0.1 %) decreased the emergence and early growth of test species in a dose-dependent manner. At 0.1 % *Callistemon* oil treatment none of the seeds of *C. benghalensis* germinated. The *Callistemon* oil not only affected the germination and early growth of weed species but also severely decreased the chlorophyll content of the test plants. The chlorophyll content decreased by ~ 71% in *C. benghalensis* in response to 0.05 % *Callistemon* oil treatment. These results strongly indicate the adverse effect of *Callistemon* oil on photosynthesis of test plants. Based on the study, it can be concluded that *Callistemon* oil possess phytotoxic potential and can be used as bioherbicide in weed management programmes.

Key words: Allelochemicals; Bioherbicide; Phytotoxicity; Weed management

Introduction

Weeds are generally referred to as unwanted plants that grow at places where not required and interfere with the growth and establishment of other useful plants. In India, Varshney and Prasad Babu (2008) reported 15-85% crop loss due to interference of weeds with crops. They serve as host for many plant diseases and pests and also adversely affect human health (Mazza *et al.*, 2014). Various mechanical, biological and chemical methods are applied for controlling weeds. Of these, mechanical and biological methods fail to provide adequate weed control and thus chemical methods are used worldwide. In fact, modern agriculture is largely based on the use of synthetic herbicides for controlling weeds obviously because their action is fast and mode of application comparatively easier. Unfortunately, during the last 20-30 years it is being realized that indiscriminate use of synthetic herbicides for controlling weeds has not only deteriorated the environment quality but has also led to development of herbicidal resistance among weeds. Due to this, efforts are being made towards the search for new / novel technologies and cultural practices for managing weeds to sustain continued crop yield (Dayan and Duke, 2014). Nowadays, natural plant products are receiving attention of plant researchers as potential bioherbicides for weed management owing to their allelopathic properties. It is primarily because these plants based products are environmentally safer, biodegradable, possesses low mammalian toxicity than the synthetic herbicides which are expensive too (Dayan *et al.*, 2012).

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Among different natural plant products, allelochemicals (chemicals responsible for allelopathy) are being explored as an important tool in weed management because of their structural and chemical diversity (Singh *et al.*, 2003). Among allelochemicals, volatile essential oils and their constituent monoterpenes are known to possess very high phytotoxicity towards a number of other plants (Muller and Muller, 1964; Singh *et al.*, 2009). Essential oils are complex mixture of mono (C_{10}) and sesquiterpenes hydrocarbons (C_{15}) along with alcohols, esters, aldehydes and ketones that determine characteristic color and odor of the donor plant. They are also used in pharmaceutical, food, cosmetic and perfume industry (Batish *et al.*, 2008).

C. viminalis (Gaertn.) G. Don., commonly known as weeping bottlebrush, is one of the very common species of *Callistemon*, extensively cultivated in the gardens, parks and road sides in different parts of the world including India (Anonymous, 1992). It has pendulous evergreen foliage with crimson red flowers. Besides, the tree is aromatic particularly its leaves and inflorescence. According to Garg and Kasera (1982), oil from *C. viminalis* exhibited anthelmintic activity against tapeworm and hookworm. The essential oil obtained from *C. viminalis* also exhibited antibacterial and antioxidant, and insecticidal activities. Owing to their selective phytotoxic effects and biodegradable nature, essential oil extracted from *C. viminalis* containing a mixture of monoterpenes offer a great deal of

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promise for future to serve as novel herbicides. Though the phytotoxic effect of compounds extracted from *C. citrinus* has been studied, yet studies pertaining to phytotoxic effect of *C. viminalis* essential oil are lacking. de Oliveira *et al.,* (2014) reported that essential oil extracted from the flowers of *C. viminalis* possess allelopathic properties. However, nothing is known about the phytotoxicity of essential oil of *C. viminalis*. Since only a few weeds grow under *C. viminalis*, it was hypothesized that plant may inhibit the growth of under-storey or nearby plants due to the release of foliar volatiles from its foliage.

Materials and Methods

Collection of plant material: Mature leaves from trees of *C. viminalis* were collected from Panjab University campus, Chandigarh, India. The material was collected in early hours to obtain maximum quantity of oil possible.

Extraction of oil: Volatile essential oil was extracted from leaves of *C. viminalis* by hydrodistillation using Clevenger's apparatus. For this 2 kg of freshly collected material was chopped into pieces and mixed with 1 L of distilled water in a container of Clevenger's apparatus fitted with a condenser. Leaves were boiled for 5 h and after cooling, oil was collected from the nozzle. The oil thus obtained was dried over anhydrous sodium sulphate and stored at 4 °C for further use.

Collection of seeds: Seeds of various weeds like Johnson grass (*Sorghum halepense* (L.) Pers), benghal dayflower (*Commelina benghalensis* L.) and billygoat weed (*Ageratum conyzoides* L.) were collected from wildly growing stands in Panjab University campus, Chandigarh whereas seeds of chinese sprangletop (*Leptochloa chinensis* (L.) Nees were collected from the agricultural fields in and around Chandigarh. These were selected because they are troublesome, economically important and are common in this region.

Growth bioassay with *Callistemon* oil in solution form: Uniform, healthy and viable seeds of test weeds were surface sterilized with 2% sodium hypochlorite for 1-2 min before imbibitions. For solution form, various concentrations (0.025, 0.05, 0.1%) of oil were prepared by dissolving oil in distilled water with the help of Tween-20. Petri dishes (15 cm) were lined with two layers of Whatman No. 1 filter paper and moistened with 10 ml of various oil concentrations as per the method of Batish *et al.,* (2004).

After 7 days, number of seeds germinated was counted in each Petri dish. Plant growth (in terms of root and shoot length) was measured with the help of scale. Leaves of test plants were taken for further determination of chlorophyll content.

Total Chlorophyll Content: Total chlorophyll content from leaves of test plants (both control and treated) was extracted in dimethyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979). The extinction value was measured at dual wavelength of 645 and 663 nm on Shimadzu UV-1800 double beam spectrophotometer using DMSO as blank. Total chlorophyll content was calculated from extinction values following Arnon`s equation (1949) and was expressed on dry weight basis, as suggested by Rani and Kohli (1991).

Statistical analysis: The statistical analysis of data was done using one-way ANOVA followed by the comparison of mean values using *post hoc* Tukey's test at $P \leq 0.05$ and finally presented as mean±SE (Standard Error) using software program SPSS (Version 16.0).

Results and Discussion

Callistemon oil reduced the germination of test plants in a concentration-dependent manner. At lower concentrations (0.025 %) of oil, very less difference in germination of treated seeds *viz. A. conyzoides* (~12%), *S. halepense* (~12%) over control was observed, however, in *L. chinensis* (~44%) and *C. benghalensis* (~44%), the germination decreased significantly ($P \leq 0.05$) compared to control (Table 1). Among the test species studied, *C. benghalensis* showed 100% inhibition in germination at 0.1 % concentration of *Callistemon* oil (Table 1). At 0.1 % *Callistemon* oil treatment, ~ 44%, ~ 45% and ~ 94% inhibition in germination was observed in *A. conyzoides*, *S. halepense* and *L. chinensis* over the control (Table 1).

Table 1: Effect of *Callistemon* oil on germination (%) of test weeds

Conc. (%)	*Ageratum conyzoides*	*Sorghum halepense*	*Leptochloa chinensis*	*Commelina benghalensis*
0	98.3±1.67a (0)	81.7±4.41a (0)	80.0 ± 5.77a (0)	95.6 ± 2.22a (0)
0.025	86.7±1.67b (11.8)	71.7±1.67a (12.2)	45.0 ± 2.89b (43.8)	53.3 ± 10.18b (44.3)
0.05	70.0±2.87c (28.8)	55.0±2.89b (32.7)	28.3 ± 3.33c (64.6)	26.7 ± 10.18c (72.1)
0.1	55.0±2.87d (44.1)	45.0±2.89b (44.9)	5.0 ± 2.89d (93.8)	-

Data presented as mean±SE.
Different alphabets represent significant difference at $P \leq 0.05$ after applying *post hoc* Tukey's test.
Values within parenthesis indicate percent decrease over control.

Table 2: Effect of *Callistemon* oil on root length (cm) of test weeds

Conc. (%)	*Ageratum conyzoides*	*Sorghum halepense*	*Leptochloa chinensis*	*Commelina benghalensis*
0	0.9±0.01a (0)	5.8±0.03a (0)	3.9 ± 0.06a (0)	4.7 ± 0.04a (0)
0.025	0.6±0.03c (33.3)	5.1±0.02b (12.1)	2.4 ± 0.05b (38.5)	3.2 ± 0.03b (31.9)
0.05	0.5±0.01d (44.4)	4.7±0.01c (19.0)	2.0 ± 0.04c (48.7)	2.2 ± 0.04c (53.2)
0.1	0.3±0.01e (66.7)	4.3±0.03d (25.9)	0.6 ± 0.04d (84.6)	-

Data presented as mean±SE.
Different alphabets represent significant difference at $P \leq 0.05$ after applying *post hoc* Tukey's test.
Values within parenthesis indicate percent decrease over control.

Table 3: Effect of *Callistemon* oil on shoot length (cm) of test weeds

Conc. (%)	*Ageratum conyzoides*	*Sorghum halepense*	*Leptochloa chinensis*	*Commelina benghalensis*
0	1.4±0.01a (0)	8.2±0.03a (0)	6.2±0.04a (0)	3.4±0.02a (0)
0.025	1.0±0.02b (28.6)	7.1±0.03b (13.4)	4.6±0.04b (25.8)	2.2±0.03b (35.3)
0.05	0.8±0.04c (42.9)	6.5±0.02c (20.7)	3.3±0.02c (46.8)	1.8±0.05c (47.1)
0.1	0.6±0.01d (57.1)	4.8±0.03d (41.5)	1.0±0.02d (83.9)	-

Data presented as mean±SE.
Different alphabets represent significant difference at $P \leq 0.05$ after applying *post hoc* Tukey's test.
Values within parenthesis indicate percent decrease over control.

Table 4: Effect of *Callistemon* oil on chlorophyll content (μg/mg DW) of test weeds

Conc. (%)	*Ageratum conyzoides*	*Sorghum halepense*	*Leptochloa chinensis*	*Commelina benghalensis*
0	9.4±0.08a (0)	7.5±0.05a (0)	10.0±0.03a (0)	10.6±0.25a (0)
0.025	8.3±0.03b (11.7)	6.7±0.06b (10.7)	8.0±0.03b (20.0)	6.0±0.14b (43.4)
0.05	7.6±0.10c (19.2)	6.5±0.03c (13.3)	8.1±0.06b (19.0)	3.1±0.13c (70.8)
0.1	5.5±0.06d (41.5)	5.7±0.06d (24.0)	5.4±0.12c (46.0)	-

Data presented as mean±SE.
Different alphabets represent significant difference at $P \leq 0.05$ after applying *post hoc* Tukey's test.
Values within parenthesis indicate percent decrease over control.

Further, the treatment of 0.025 % *Callistemon* oil reduced the root length by ~ 33%, 12%, 39% and ~ 32% in *A. conyzoides*, *S. halepense*, *L. Chinensis*, *C. benghalensis,* respectively over the control (Table 2). In response to 0.1 % *Callistemon* oil, ~ 67%, ~ 26% and ~ 85% and 100% reduction in root length was recorded in *A. conyzoides*, *S. halepense*, *L. chinensis* and *C. benghalensis*, respectively, compared to the control (Table 2). In general, the shoot length of all the test plants decreased with increase in concentration of oil. In control seedlings of *A. conyzoides*, *S. halepense, L. chinensis* and *C. benghalensis,* the coleoptile length was 1.4±0.01, 8.2±0.03, 6.2±0.04 and 3.4±0.02 cm, respectively. Treatment of 0.025% oil reduced the shoot length by ~ 29%, ~ 13%, ~ 26% and ~ 35% in *A. conyzoides*, *S. halepense*, *L. chinensis* and *C. benghalensis*, respectively, compared to control (Table 3). At 0.1 % oil treatment, *A. conyzoides*, *S. halepense*, *L. chinensis* and *C. benghalensis* showed ~ 57%, ~ 42%, ~ 84% and 100 % inhibition in shoot length.

The growth retardatory effects of *Callistemon* oil on the test plants is in agreement with earlier studies reporting inhibitory effect of volatile oils from aromatic plants on germination and growth of weeds (Singh *et al.*, 2009; Rolli *et al.*, 2014). Recently, Ghnaya *et al.,* (2016) investigated the herbicidal activity of *Tetraclinis articulata* (Vahl) Mast. essential oil on *Sinapis arvensis* L. and *Phalaris canariensis L.* The results indicated complete inhibition of germination in *S. arvensis* at 4 μl/ml concentration of oil (Ghnaya *et al.*, 2016). The exact mechanism for the reduced germination and early growth of weeds is not known but it could be due to the inhibitory effect of volatiles on the cell division and DNA synthesis of growing root tips (Romagni *et al.*, 2000; Nishida *et al.*, 2005). The effect of *Callistemon* oil was more on root growth as compared to shoot growth which might be attributed to the effect of volatile oils on proliferation of root apical meristem cells (Singh *et al.*, 2006) that results in various physiological changes in the plant. The observed inhibitory activity of essential oils in the study may be attributed to the synergistic effect of the constituents in *Callistemon* oil.

Further, the chlorophyll content declined upon exposure to different concentrations of *Callistemon* oil. At 0.1 % of oil, the chlorophyll content decreased by ~ 42%, ~ 24%, 46% and 100% compared to the control, in *A. conyzoides*, *S. halepense*, *L. Chinensis* and *C. benghalensis (*Table 4). Among the weeds, *C. benghalensis* was the most affected one, followed by *L. chinensis*, *A. conyzoides* and *S. halepense*. These observations are in conformity with earlier reports that volatiles reduce photosynthetic pigments and thus affect the photosynthetic activity in the plants (Batish *et al.*, 2004; Kaur *et al.*, 2010). However, whether the observed reduction in chlorophyll content was due to decreased synthesis or enhanced degradation of chlorophyll is not known (Yang *et al.*, 2002).

Conclusions

The present study therefore concludes that *Callistemon* oil inhibit germination and early growth of weeds by altering the photosynthetic and respiratory metabolism of plants and therefore holds good potential to be used as bioherbicide in the future.

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