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Phytotoxicity of volatile oil from *Eucalyptus citriodora* against some weedy species

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Abstract: *A study was undertaken to explore the phytotoxicity of volatile essential oil from Eucalyptus citriodora Hook. against some weeds viz. Bidens pilosa, Amaranthus viridis, Rumex nepalensis, and Leucaena leucocephala in order to assess its herbicidal activity. Dose-response studies conducted under laboratory conditions revealed that eucalypt oils (in concentration ranging from 0.0012 to 0.06 %) greatly suppress the germination and seedling height of test weeds. At 0.06 % eucalypt oil concentration, none of the seed of test weeds germinated. Among the weed species tested, A. viridis was found to be the most sensitive and its germination was completed inhibited even at 0.03%. Not only the germination and seedling growth, even the chlorophyll content and respiratory activity in leaves of emerged seedlings were severely affected. In A. viridis chlorophyll content and respiratory activity were reduced by over 51% and 71%, respectively, even at a very low concentration of 0.06%. These results indicated an adverse effect of eucalypt oils on the photosynthetic and energy metabolism of the test weeds. A strong negative correlation was observed between the observed effect and the concentration of eucalypt oil. Based on the study, it can be concluded that oil from E. citriodora possess strong inhibitory potential against weeds that could be exploited for weed management.*

Key words: *Bioherbicides, Chlorophyll content, Dose-response studies, Respiratory activity, Seedling growth, Weed management*

Introduction

Weeds are unwanted and undesirable plants that interfere with utilization of land and water resources and thus, adversely affect human welfare. In croplands and forests, weeds compete with desired and beneficial vegetation, reducing the yield and quality of produce. Global economic losses due to weeds are enormous and a huge amount of money is spent to control them (Zimdahl, 1999). Though the control of weeds can be achieved by several methods, yet the use of synthetic herbicides is quite common and effective. Unfortunately, the indiscriminate use of synthetic herbicides during the last three decades has resulted in various toxicological effects on the environment and living organisms including humans. Moreover, their continuous use has resulted in evolution of new weed biotypes with herbicidal resistance. To overcome these problems, efforts are being made world over to find out alternative means, which are not only eco-friendly but, also cost effective and bioefficaceous. In this direction, screening of natural plant products depicting herbicidal and pesticidal potential has gained momentum since they are not only biodegradable and possess novel molecular target sites but also have diverse chemical nature with no or less halogen atoms and heavy metals (Dayan *et al*., 1999; Duke *et al*., 2002).

Among the natural plant products, volatile essential oils-the constituents of aromatic plants, are known to possess relatively high phytotoxicity (Singh *et al*., 2003) and degrade quickly in the environment (Beuchat, 2001). Terpenoids, particularly monoterpenes and sesquiterpenes, are the main components of essential oils and are responsible for the inhibitory activity of these oils. *Eucalyptus* (family Myrtaceae) species are well-known for their essential oils that find profuse use in medicines, perfumery and as flavouring agents. Among various species, lemon-scented eucalypt (*Eucalyptus citriodora* Hook.) is well-known for its antimicrobial (Dellacassa *et al*., 1989), antifungal (Ramezani *et al*., 2002), insecticidal (Isman, 2000), and nematicidal (Pandey *et al*., 2000) activities. Singh and Kohli (1992) reported that plantations of *E. citriodora* have very little vegetation under their canopy and around them and it is due to the release of oil vapours from the trees, which move downwards and affect the adjoining vegetation. However, little has been done to further explore their phytotoxic potential against weeds. Therefore, the present investigation was undertaken to assess the phytotoxicity of eucalypt oil against some weeds with a view to explore them as a bioherbicide for weed management.

Materials and Methods

Extraction of oil: Eucalypt oil was extracted using Clevenger apparatus from healthy and mature freshly collected leaves of lemon-scented eucalypt *(Eucalyptus citriodora* Hook.) trees of nearly 25 year age growing in Botanical Garden, Panjab University, Chandigarh, India. Leaves (250g) were chopped and mixed with 1 liter distilled water in a 2 liter round bottom flask and fitted with condenser. The mixture was boiled for 3hr and oil was collected from the nozzle of the condenser, dried under sodium sulphate and stored at 4 °C for further use.

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Procurement of test material: For the study, seeds of hairy beggar's tick (*Bidens pilosa* L.), green amaranth (*Amaranthus viridis* L.) and Himalayan dock (*Rumex nepalensis* Spreng.) were collected from wildly growing strands of these weeds in and around Panjab University Campus, Chandigarh, India. Besides these three weeds, lead tree / subabul [*Leucaena leucocephala* (Lam.) de Wit.] was also selected for the present study as the seeds from the mature trees fall down in plenty, germinate and it assumes a weedy proportion. Its seeds were collected from nearly 8 year old trees, pretreated with concentrated H_2SO_4 for 2 min for scarification, then washed with distilled water three times and used for further studies. Seeds of all the test species were surface sterilized with 1% sodium hypochlorite for 2 min, washed with distilled water and stored for further use.

Dose-response studies: To test the inhibitory effect of oil, different concentrations of eucalypt oil (0.0012, 0.003, 0.006, 0.012, 0.03, and 0.06%) were used and their effect was studied on germination and seedling growth of test weeds. Twenty seeds of each weed type (10 in case of *L. leucocephala*) were placed on a Whatman no. 1 filter circle moistened with 7 ml of different concentrations of eucalypt oil. A similar treatment with water only served as control. For each concentration, five replications were maintained. All the petridishes were kept in a growth chamber maintained at 16/8 light/dark period, 25± 2 °C temperature, 80% relative humidity and 150 µ mole $m^{-2} s^{-1}$ photosynthetic photon flux density. After 7 days, germinated seeds were counted, seedling height was measured and amount of total chlorophyll and cellular respiration in leaves was determined.

Chlorophyll estimation: Total chlorophyll from 25 mg of leaves (control or treated) was extracted in 4 ml of Dimethyl sulphoxide (DMSO) following the method of Hiscox and Israesltam (1979). The extinction value of chlorophyll thus recovered in DMSO was measured at dual wavelength of 645 and 663 nm on Shimadzu Spectrophotometer using DMSO as blank. Total chlorophyll content was calculated from extinction value following the equation of Arnon (1949) and expressed on dry weight basis as suggested by Rani and Kohli (1991). Values on dry weight equivalents were calculated by placing same amount of tissue in an oven at 80°C for 24 hr.

Cellular respiration: The cellular respiration or cell survival value was determined indirectly using 2,3,5-triphenyl tetrazolium chloride following the method of Steponkus and Lanphear (1967) wherein the red formazan formed traps the oxygen released through respiratory chain and thus respiration can be measured indirectly. The absorbance was read at 530 nm and the values were expressed with respect to control.

Statistical analysis: Experiments were conducted in a completely randomized manner with five replicates. These were repeated and mean data of two experiments is presented. Data were subjected to one-way analysis of variance and significance of treatments from control was tested at 1 and 5% level of significance applying Dunnett's test. Further, data were also subjected to determination of correlation coefficient between eucalypt oil concentration and the observed response. The statistical analysis was performed using SPSSPC software version 10.0.

Results and Discusssion

The results show that eucalypt oil reduced the germination of all the test weeds (Table 1). At lower concentrations, the effect of oil on percent germination was lesser and it increased with increasing concentration of eucalypt oil. At 0.06% eucalypt oil treatment, a complete inhibition of germination of all the test weeds was observed. The response of weed seeds to eucalypt oil was differential which may be due to the variable seed size and genetic variability. Maximum inhibition was noticed in *A. viridis* where none of the seed germinated even at 0.03% eucalypt oil concentration. In contrast, the germination reduction ranged from nearly 21-28% in the other three weeds at 0.03% concentration of eucalypt oils. In terms of germination, *B. pilosa* was the least sensitive plant (Table 1). A strong negative correlation coefficient value was observed between eucalypt oil concentration and percent germination. Based on the dose-response studies, LC_{50} values (at which germination is inhibited by 50%) were calculated to be 0.017%, 0.040%, 0.037% and 0.039% for *A. viridis, R. nepalensis, L. leucocephala* and *B. pilosa*, respectively. LC_{50} values can serve as important tools for further determination of the mechanism of action of these oils. The observed inhibitory effect of eucalyptus volatile oils on seed germination is not new

Table - 1: Effect of eucalypt oil on the percent germination of the test weed species. *r* represents correlation coefficient values

Concentration (%)	*B. pilosa*	*A. viridis*	*R. nepalensis*	*L. leucocephala*
Control (0)	100 ± 0	100 ± 0	100 ± 0	100 ± 0
0.0012	96.2 ± 2.15	99.3 ± 0.48	98.2 ± 1.12	90.8 ± 1.47*
0.003	93.1 ± 1.96*	98.3 ± 0.78	96.4 ± 2.16	88.0 ± 2.61*
0.006	90.4 ± 2.34*	83.2 ± 1.64*	92.8 ± 3.45*	78.7 ± 3.29**
0.012	87.3 ± 2.49*	48.6 ± 2.38**	83.9 ± 2.49*	75.1 ± 2.08**
0.03	79.4 ± 3.15**	0[a]	71.3 ± 3.24**	72.4 ± 2.34**
0.06	0	0	0	0
r-value	– 0.951	– 0.897	– 0.978	– 0.954

± represents standard deviation; [a] no germination. * and ** represent significance from respective control at $p<0.05$ and 0.01, respectively, applying Dunnett's test

as essential volatile oils from a number of species are well-known as germination inhibitors (Dudai *et al*., 1999; Kohli, 1990; Mao *et al*., 2004; Muller and Muller, 1964; Singh *et al*., 1991; Vokou, 1999).

The oil not only reduced the percent germination but also the seedling growth of test weeds. There was a drastic reduction in seedling height of germinated seeds with increasing concentration of oil (Table 2). At 0.012% concentration, there was nearly 67% reduction in seedling height of *A. viridis*, whereas nearly 11% reduction was observed in *L. leucocephala*. Here also, a strong and negative correlation was observed between concentration and observed seedling height. The reasons for reduced seedling height could not be known, but can be attributed to inhibition / reduction of mitotic activity of growing seedlings. Vaughn (1991) reported that oils from cinnamon (*Cinnamomum zeylanicum* Blume) and red thyme (*Thymus vulgaris* L.) inhibit potato sprouts by killing its meristematic cells. Earlier, Lorber and Muller (1976) reported that volatile substances from purple sage (*Salvia leucophylla* Greene) disrupt the mitotic activity and reduce root tip development in onion roots.

Table - 2: Effect of eucalypt oil on seedling height (cm) of test weeds

Concentration (%)	*B. pilosa*	*A. viridis*	*R. nepalensis*	*L. leucocephala*
Control (0)	9.1 ± 0.13	7.7 ± 0.98	5.9 ± 0.25	15.0 ± 0.71
0.0012	8.0 ± 0.06	4.4 ± 0.18**	5.8 ± 0.11	10.5 ± 0.43*
0.003	7.9 ± 0.18	3.6 ± 0.14**	5.7 ± 0.35	9.8 ± 0.30*
0.006	7.0 ± 0.13*	3.2 ± 0.05**	5.4 ± 0.16*	8.8 ± 0.16*
0.012	5.6 ± 0.34**	2.5 ± 0.03**	5.2 ± 0.15*	8.3 ± 0.14**
0.03	3.4 ± 0.13**	0[a]	3.1 ± 0.11**	7.7 ± 0.42**
0.06	0	0	0	0
r-value	– 0.983	– 0.791	– 0.997	– 0.910

± represents standard deviation; [a] seedling height was zero, since there was no germination at these concentrations. * and ** represent significance from respective control at p<0.05 and 0.01, respectively, applying Dunnett's test, *r* - represent corelation coefficient values

Table - 3: Effect of eucalypt oil on the chlorophyll content (µg/mg dry weight) in the test weed species. *r* represents correlation coefficient values. Data in parenthesis represent percent reduction from control

Concentration(%)	*B. pilosa*	*A. viridis*	*R. nepalensis*	*L. leucocephala*
Control (0)	9.80 ± 0.32(0)	7.91 ± 0.43(0)	6.94 ± 0.47(0)	7.69 ± 0.41(0)
0.0012	8.52 ± 0.29*(13.1)	6.64 ± 0.19*(16.1)	6.10 ± 0.25(12.1)	4.78 ± 0.39*(37.9)
0.003	8.24 ± 0.14*(15.9)	5.19 ± 0.37*(34.4)	5.75 ± 0.42*(17.1)	3.71 ± 0.27**(51.7)
0.006	7.60 ± 0.37*(22.4)	3.82 ± 0.26**(51.7)	4.61 ± 0.21*(33.6)	3.18 ± 0.23**(58.6)
0.012	5.78 ± 0.28**(41.0)	2.21 ± 0.13**(72.1)	4.16 ± 0.34**(40.1)	2.57 ± 0.14**(66.6)
0.03	3.72 ± 0.13** (62.0)	0[a](100)	4.07 ± 0.40**(41.4)	1.48 ± 0.42**(80.8)
0.06	0(100)	0(100)	0(100)	0(100)
r-value	– 0.980	– 0.830	– 0.950	– 0.800

± represents standard deviation; [a] no chlorophyll, since there was no germination at these concentrations. * and ** represent significance from respective control at p<0.05 and 0.01, respectively, applying Dunnett's test

Table - 4: Effect of eucalypt oil on the percent cellular respiratory activity in the test weed species. *r* represents correlation coefficient values

Concentration (%)	*B. pilosa*	*A. viridis*	*R. nepalensis*	*L. leucocephala*
Control (0)	100 ± 0	100 ± 0	100 ± 0	100 ± 0
0.0012	76.3 ± 3.49*	57.3 ± 4.21**	90.4 ± 1.37*	92.7 ± 1.57*
0.003	70.2 ± 1.24*	39.6 ± 2.34**	86.2 ± 1.51*	84.5 ± 2.01*
0.006	63.1 ± 1.39**	28.5 ± 2.98**	79.2 ± 2.19*	69.3 ± 2.87*
0.012	47.9 ± 2.49**	19.3 ± 1.59**	67.9 ± 2.37*	60.7 ± 1.76**
0.03	44.7 ± 1.29**	0[a]	60.0 ± 2.49**	57.3 ± 2.18**
0.06	0	0	0	0
r-value	– 0.931	– 0.716	– 0.979	– 0.959

± represents standard deviation; [a] no respiration, since there was no germination at these concentrations. * and ** represent significance from respective control at p<0.05 and 0.01, respectively, applying Dunnett's test

Further, the chlorophyll content in the emerged seedlings was drastically reduced compared to control (Table 3). At 0.012% eucalypt oil concentration, chlorophyll content was reduced by about 72% in *A. viridis* and nearly 40% in *B. pilosa* and *R. nepalensis.* In *L. leucocephala* the chlorophyll content was reduced by over 80% in response to 0.03% eucalypt oil (Table 3). The reduction in chlorophyll content affects the photosynthetic machinery of the plants. Though the mechanism of chlorophyll reduction could not be ascertained, yet it is speculated that oil might be interfering with metabolism of chlorophyll either by enhancing chlorophyll degradation or reducing synthesis or both.

Likewise, a drastic reduction was observed in the respiration of emerged seedlings in response to eucalypt oils (Table 4). In response to 0.012% eucalypt oil treatment, a reduction of nearly 80% in cellular respiration was observed in *A. viridis*. In contrast, at same concentration, the respiratory activity was reduced by nearly 52% in *B. pilosa*. At 0.03% eucalypt oil respiration was reduced by nearly 55% in *B. pilosa*, 40% in *R. nepalensis* and 43% in *L. leucocephala.* The loss of respiration may alter the energy metabolism of plants and thus their growth. Abrahim *et al*. (2000) reported that monoterpenes, the constituents of volatile oils, act as uncouplers of oxidative phosphorylation and thus affect respiration. As regards eucalypt oil, its monoterpenes have been observed to drastically affect the cellular respiration in plants (Singh *et al*., 2002).

The present study therefore concludes that volatile oils from *E. citriodora* inhibit the germination and growth of weedy species by affecting the photosynthetic and respiratory metabolism. Such studies provide clues for the possible utilization of eucalypt oil as bioherbicides in future.

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