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Herbicidal Potential of *Callistemon viminalis* Essential Oil against *Echinochloa crus-galli* L., *Amaranthus viridis* and *Phalaris minor*

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Abstract

Experimental studies were carried out under *invitro* conditions to examine the effect of *Callistemon viminalis* essential oil (EO) against the *Echinochloa crus-galli* L., *Amaranthus viridis* and *Phalaris minor*. EO composition was analysed by gas chromatography/mass spectrometry, revealing that EO was rich in monoterpenes and sesquiterpenes like eucalyptol, D-limonene, α-terpineol and caryophyllene oxide. EO applied in the concentration range of 0.5, 1, 2 and 4 µl against *Amaranthus viridis, Echinochloa crus-galli* L. and *Phalaris minor* drastically affected the germination of all test plants and inhibited root and shoot development. Also, not only seedling growth, even the chlorophyll content also reduced appreciably. This led to the demonstration that *Callistemon viminalis* EO was negatively affecting the photosynthetic process. Upregulation of antioxidant enzymes activities indicated that these enzymes were providing protection against *Callistemon viminalis* EO induced stress. *Amaranthus viridis* was reported to be more sensitive in comparison to *Echinochloa crus-galli* L. and *Phalaris minor*.

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Introduction

Agricultural practices depend on the various factors like rain fall, weather conditions, soil fertility and the presence of pests such as weeds. Weeds are unwanted plants that compete with crop plants and affect their quality and quantity.[1,2] Moreover, weeds are also known to deteriorate soil, pollute water and cause disease in the nearby plants due to the release of poisonous chemicals. Modern agriculture practices for the control of weeds are largely dependent on the use of chemical herbicides. However, synthetic herbicides are associated with toxic effects on plants, animals and human beings.[3] Limitations of synthetic herbicides can be overcome by the development of bioherbicides. Therefore, efforts are being made to develop herbicides from

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natural products such as Essential Oils (EOs) from aromatic plants[4,5]. *Callistemon viminalis* plant is a good option for EO extraction since it can survive in dry season and poor soil, and provides large biomas[6]. *Amaranthus viridis, Echinochloa crus-galli* L. and *Phalaris minor* weeds are responsible for huge economic losses[7]. Keeping the above facts in the mind, the experimental work was designed to assess the phytotoxic potential of *C. viminalis* EO against *E. crus-galli* L., *P. minor* and *A. viridis* for the exploration of its bio herbicidal nature.

Material and Methods

About 700g leaves of test plant i.e., *C. viminalis* were collected from the campus of Central University of Punjab, Bathinda (30.1700° N, 76.4500° E). Seeds of test weeds (*E. crus-galli L., P. minor* and *A. viridis*) were procured from Punjab Agriculture University Ludhiana, Punjab, India.

Extraction of EO

EO from leaves of C. *viminalis* was extracted for 3 hrs by steam distillation method using Clevenger's apparatus and stored at 4°C until evaluation for chemical composition and biochemical studies.

Composition of EO

Chemical composition along with the identification of the major compounds present in the EOs was studied using Gas Chromatograph coupled with Mass Spectrophotometer (GC-MS) (Shimadzu QP 2010 Mass Spectrophotometer). The following conditions were applied during the GC-MS.

Detector: Flame Ionization Detector (FDI)

Column: Stabil Wax with 30 × 0.25 mm length and 0.25 µm film thickness

Carrier Gas: Helium

Oven temperature: 40°C (held isothermally for 4 min) and increased at a rate of 4°C per min up to 220°C (isothermally for 5 min).

Further analysis was done on mass spectrometer and linear velocity of 38.5 cm sec^{-1}. The mass spectra were screened in the range of m/z 40-600 amu. The compounds identification was carried out by comparing Kovats/retention indices (RI) with reference to C_7-C_{30}series of *n*-alkanes (Supelco, Bellefonte, PA, USA). Some compounds were searched using reference book[8].

Phytotoxicity of EOs

Seeds of test weeds were imbibed in distilled water for 12 hrs and grown in Petri dishes (Ø= 15 cm) lined with filter paper. EO (0.5, 1, 2, and 4 µl) was spread on the inner side of lid of Petri dish and sealed with Parafilm to avoid volatilization. A similar set up with distilled water served as control. The whole set up was kept for seven days in growth chamber under controlled conditions. After seven days, the germinated seedlings of control as well as treatments were assessed for several parameters such as percent germination, seedling length and dry weight[9]. Chlorophyll content[10,11] and cellular respiration were also determined[12].

Assays for Anti-Oxidant Enzymes

Enzyme Extraction

Tissue homogenized in phosphate buffer (0.1 M) along with centrifugation for 15 minutes at 10,000 rpm was stored at 4°C for biochemical studies.

Antioxidant Enzyme Activities

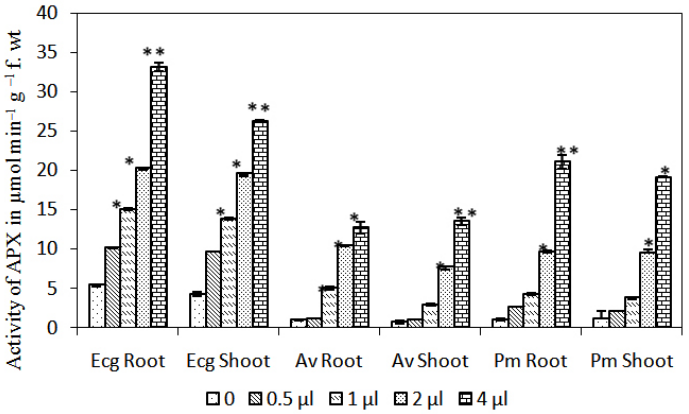
Ascorbate peroxidase (APX; EC 1.11.1.11): The reaction mixture required for the assessment of APX consisted of PO_4^{3-} buffer (25 mM, pH 7.0), EDTA (0.1 mM), ascorbate (0.25 mM), H_2O_2(1 mM) and enzyme extract (0.2 ml). Decline in absorbance of reaction mixture was measured at 290 nm[13]. Extinction coefficient of 2.8 mM^{-1} cm^{-1} was used for measuring the enzyme activity. Amount of enzyme that was required to oxidize 1 mM ascorbate min^{-1} was defined as one enzyme unit.

Guaiacol Peroxidase (GPX; EC 1.11.1.7)

Oxidation of guaiacol was used for measuring the activity of GPX[14]. The reaction mixture required for the determination of GPX consisted of PO_4^{3-} buffer (25mM, pH 7.4), guaiacol (0.05%), H_2O_2(1mM), EDTA (0.1mM) and enzyme extract (0.2ml). Extinction coefficient of 26.6 mM^{-1}cm was used for calculating the enzyme activity. Increase in absorbance of the reaction mixture upon the addition of enzyme extract was measured at 470nm and expressed as EU mg^{-1} protein. Amount of enzyme required to catalyze the oxidation of 1mM guaiacol min^{-1} was defined as one enzyme unit.

Calculation and Statistics

Experiments were conducted in a completely randomized block design. Every experiment was repeated two times. For each treatment three replicates were maintained. Experimental data were subjected to one way ANOVA with Tukey's test.



The error bars show the mean±SE and the asterisk signs show the significant difference on applying post hoc Tukey's test at p≤0.05. One asterisk p≤0.05, two asterisks P ≤0.01

Fig. 1: Activity of APX in µmol min^{-1} g^{-1} f. wt with the increasing amount of EOs from 0-4 µl in *E. crus-galli* (Ecg), *P. minor* (Pm) and *A. viridis* (Av).

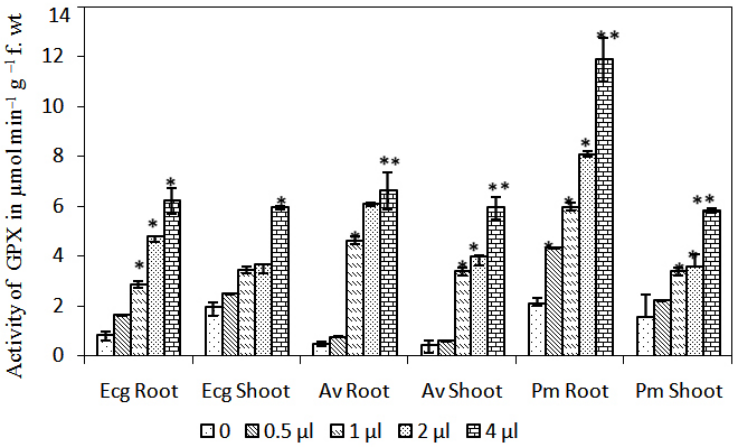
Table 1: Effect of Essential oil from *Callistemon viminalis* on percent germination and dry weight of *E. crus-galli*, *P. minor* and *A. viridis*.

	Effect of EOs on Percent Dry weight , Germination and Seedling length								
	E. crus-galli			*P. minor*			*A. viridis*		
Volume of oil (µl)	% Germination	% Dry weight	Seedling length (cm)	% Germination	% Dry weight	Seedling length in cm	% Germination	% Dry weight	Seedling Length (cm)
0	100± 0.024[a]	100± 0.056[a]	20± 0.018[a]	100± 0.001[a]	100± 0.021[a]	19± 0.012[a]	100± 0.001[a]	100± 0.021[a]	12± 0.066[a]
0.5	81.37± 0.054[b]	74.44± 0.019[b]	15± 0.053[b]	77.77± 0.023[b]	72± 0.034[b]	12± 0.024[b]	69± 0.054[b]	72± 0.034[b]	8.6± 0.039[b]
1.5	79.41± 0.045[b]	62.22± 0.046[c]	8.6± 0.042[c]	69.44± 0.023[c]	65.55± 0.023[c]	8.2± 0.03[c]	54.55± 0.021[b]	65.55± 0.023[c]	6.3± 0.013[c]
2.5	68.74± 0.020[c]	56.22± 0.067[d]	3.6± 0.028[d]	61.11± 0.033[d]	51.11± 0.022[d]	5.1.± 0.042[d]	44.31± 0.089[b]	51.11± 0.022[d]	2.3.± 0.030[d]
5	44.98± 0.063[d]	37.77± 0.034[e]	2.1.± 0.052[e]	46.66± 0.067[e]	38.88± 0.076[e]	1.8.± 0.052[e]	17.24± 0.027[c]	38.88± 0.017[e]	1.2.± 0.023[e]

Data is represented as mean ± standard error and different letters (a-e) within a column represent significant difference among various treatments and control, according to one-way ANOVA followed by post hoc Tukey's test at P<0.05.

Results and Discussion

GCMS analysis showed that EO was composed of 22 compounds constituting nearly 99.90% of volatile oil. The average yield of EO was calculated 0.853 ± 0.009% (w/w). Major compounds identified were 1, 8-cineole (64.5%), α-terpineol (19.7%), + (-)-limonene (4.7%), trans-geraniol (2.0%) and linalool (1.43%). The oil was comprised of monoterpene hydrocarbons (50%), oxygenated monoterpenes (33.33%), sesquiterpene hydrocarbons (11.55%) and oxygenated sesquiterpenes (5.5%). The similar composition and EOs yield was also reported by Srivastava et al.,[6] plant sample collected from Indian sub-continent. Further Oyedeji *et al.*,[15] and de Oliveira *et al.*,[21] also reported 0.9% and 1.42 % yields of EOs respectively, from the plants collected from African subcontinent. The variations in the yield are mainly due to the changes in the geographical and climatic conditions.



The error bars show the mean±SE and the asterisk signs show the significant difference on applying post hoc Tukey's test at p≤0.05. One asterisk p≤0.05, two asterisks P ≤0.01

Fig. 2: Activity of GPX in µmol min^{-1} g^{-1} f. wt with the Increasing the amount EOs from 0-4 µl in *E. crus-galli*(Ecg), *P. minor*(Pm) and *A. viridis*(Av).

Table 2: Effect of EOs on Chlorophyll and Percent Cellular Respiration

Volume of EOs (µl)	Effect of EOs on Percent Chlorophyll Content and Cellular Respiration					
	E. crus-galli L.		*P. minor*		*A. viridis*	
	% Chlorophyll	% Respiration	% Chlorophyll	% Respiration	% Chlorophyll	% Respiration
0	100.00±0.014^{a}	100±0^{a}	100 ±0.051^{e}	100±0.004^{a}	100	100±0.004^{a}
0.5	90.78±0.061^{b}	88.4±0.038^{b}	64.34 ±0.034^{b}	79.41±0.045^{b}	68.97±0.019^{b}	47.51±0.047^{b}
1.5	55.94±0.021^{c}	46.8±0.049^{c}	54.39 ±0.052^{c}	51.53±0.013^{b}	48.76±0.045^{c}	19.53±0.073^{b}
2.5	43.53±0.18^{d}	26.6±0.090^{d}	41.23 ±0.057^{d}	37.77±0.034^{e}	36.15±0.017^{d}	7.77±0.034^{e}
5	34.33±0.062^{e}	14±0.021^{e}	39.45.±0.02^{e}	16.94±0.021^{c}	24.24±0.021^{e}	-------

Data is represented as mean ± standard error and different letters (a-e) within a column represent significant difference among various treatments and control, according to one-way ANOVA followed by post hoc Tukey's test at P<0.05.

C. *viminalis* EO was observed to be phytotoxic against test weeds as it was affecting physiological parameters like inhibition of germination, decrease in seedling growth and dry weight as shown in Table 1.Previously, 1, 8-cineol, α-terpineol, limonene, linalool and α- and β- pinene were also reported as major constituents of EOs[15]. These constituents were also reported as potent phytotoxic compounds by previous researchers. Our finding on seed germination and other physiological parameters were having homology with the previous studies[16]. Maximum effect on germination of *A. viridis* as compared to *P. minor* and *E. crus galli* L. was probably due to small sized seeds and smooth seed coat of *A. viridis*[17]. Chlorophyll content and cellular respiration in seedlings of test weeds was reduced due to exposure of *C. viminalis* EO, showing that EO was affecting the energy metabolism of weed plants (Table 2).Reduction in chlorophyll content and cellular respiration in the treated seedlings was due to the alteration of leaf diffusibility, transpiration rate and stomata aperture that leads to a change in the rate of photosynthesis[18]. Decrease in cellular respiration of test plants was due to the oxidative damage of mitochondria that is responsible for ATP production. As a result energy supply gets hindered and plant growth gets retarded.[19] EO induced stress environment for the test plants that led to the enhancement in the activity of antioxidant enzymes so as to combat the stress. With an increase in the concentration of EO, there was a progressive elevation in the activities of APX and GPX. In case of *E. crus-galli* L., APX activity elevated from 5-33 μmol min^{-1} g^{-1} f. wt and 4-28 μmol min^{-1} g^{-1} f. wt for roots and shoots, respectively and the GPX activity was found in the range of 2-6 μmol min^{-1} g^{-1} f. wt both in roots and shoots (Fig 1 and Fig 2). In case of *A. viridis,* the activities of both the test enzymes were at their highest for 1-4 μl of EO treatment (Fig 1 and Fig 2). For *P. minor*, APX activity was reported to be 2-22 mol min^{-1} g^{-1} f. wt and 3-18 μmol min^{-1} g^{-1} f. wt. in roots and shoots, respectively, for 0.5-4 μl EO treatment. GPX activity was observed to be 2-12 μmol min^{-1} g^{-1} f. wt and 2-6 μmol min^{-1} g^{-1} f. wt. for 0.5-4 μl, both in roots and shoots, respectively (Fig 1 and 2). Up regulation of antioxidant enzymes activities was reported due to the generation of reactive oxygen species (ROS) in response to the EOs dose. Elevation in antioxidant enzyme activities suggested the induction of oxidative stress in tissues[20].

Conclusion

Experimental study led to the conclusion that *C. viminalis* EO could be employed as potential herbicide for weed management in agro ecosystems.

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Conflicts of Interests

Authors declare no conflicts of interests.

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