Phytotoxicity of Citronellol Against *Amaranthus Viridis* L.

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Abstract— **A study was undertaken to explore the phytotoxicity of citronellol, a volatile monoterpene found in *Eucalyptus citriodora* Hook. and many other aromatic plants, against the weed *Amaranthus viridis* L. in order to assess its herbicidal activity. Dose-response studies conducted under laboratory conditions revealed that citroellol (in concentrations ranging from 0.1µL to 2 µL) greatly suppresses the germination and seedling height of the test weed. At 2 µL concentration of citronellol, none of the seeds of the test weed germinated. At 1 µL concentration of citronellol, the radicle height of the test weed was reduced to a mere 28% and the plumule height was reduced to about 40% compared to control. Not only the germination and seedling growth, even the chlorophyll content and respiratory activity in the leaves of emerged seedlings of *A. viridis* were severely affected. The chlorophyll content and respiratory activity were reduced by about 30% and 60%, respectively, even at a very low concentration of 1 µL. These results indicated an adverse affect of citronellol on the photosynthetic efficiency and energy metabolism of the test weed. A strong negative correlation was observed between the concentration of citronellol and the observed effect. Based on the study, it can be concluded that citronellol possesses strong inhibitory potential against weeds that could be exploited for weed management.**

***Index Terms*—Bio-herbicides, Chlorophyll content, Dose-response studies, Respiratory activity, Seedling growth, Weed management.**

I. INTRODUCTION

Weeds are unwanted and undesirable plants that adversely affect human welfare by interfering with the utilization of land and water resources. In agricultural lands and forests, weeds compete with desirable vegetation and thus reduce the yield and quality of the produce. Enormous economic losses are incurred globally because of weeds and a huge amount of money is spent to control them [17]. Though several methods are employed for the control of weeds, yet the use of synthetic herbicides is quite common and effective. Unfortunately however, their continuous use during the last four decades has resulted in various toxicological effects on the environment and living beings including humans. Moreover, their indiscriminate use has resulted in the evolution of new weed biotypes possessing herbicidal resistance. Thus, efforts are being made the world over to find out some better alternatives, which are eco-friendly, cost effective and bio-efficaceous. In this direction, screening of natural plant products possessing herbicidal and pesticidal properties has gained momentum since these are bio-degradable, possess novel molecular target sites and also have diverse chemical nature with no or less halogen atoms and heavy metals [5], [6].

Among various classes of natural plant products, volatile monoterpenes are quite significant owing to their high phytotoxicity and quick bio-degradability [4]. For example, [11] reported that plantations of *Eucalyptus citriodora* have little vegetation in their vicinity which is due to the release of volatile oil vapours released from the trees. The germination and seedling growth in *Amaranthus viridis* has been reported to be inhibited by limonene, a volatile monoterpene [13]. Citronellol is also a volatile monoterpene found in aromatic plants like *Eucalyptus citriodora, E. globulus, Ocimum bacilicum, Zingiber officinale* and many others but little has been done to explore its allelopathic ability against weedy species. In the present investigation, the phytotoxic effectof citronellol against the weedy species – *Amaranthus viridis* L. was investigated. Thus, the objective of the present study is to explore the possible potential of citronellol as an environment-friendly bio-herbicide for the management and control of obnoxious weeds.

II. MATERIALS AND METHODS

A. Collection of material

Seeds of *Amaranthus viridis* L. were collected from locally growing wild stands in the outskirts of Chandigarh. Citronellol of technical grade was procured from Lancaster, U.K.

B. Bioassay studies

Fifty seeds of *Amaranthus viridis* L. after proper imbibition for 8 h were equidistantly placed on properly moist single layer of Whatman filter paper No. 1 in a 15 cm diameter Petri dish. Treatment of citronellol was given in concentrations ranging from 0.1, 0.5, 0.7, 1, 2 and 5 µL and Petri dishes were properly sealed with a cellotape. A similar set up but without the treatment of citronellol served as control. For each treatment, 5 replicates were maintained. The entire set up was placed in an environmentally controlled seed germination chamber at 25 ± 2 °C and 75 ± 2 % relative humidity with photoperiod of 16 / 8 h day / night. After 7 days, number of seeds that germinated was counted and redicle length, plumule length and seedling dry weight were measured.

C. Estimation of chlorophyll content

Total chlorophyll was extracted from 25 mg of leaves (control or treated) in 4 ml of Dimethyl sulphoxide (DMSO) following the method of [7]. The extinction values of chlorophyll thus recovered in DMSO was measured at dual wavelengths of 645 nm and 663 nm on Shimadzu Spectrophotometer using DMSO as blank. Total chlorophyll content was calculated from extinction values following the equation of [2] and expressed on dry weight basis as per [9]. Values on dry weight equivalents were calculated by placing same amount of tissue

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in an oven at 80°C for 24 hr.

D. Determination of cellular respiration

The cellular respiration was determined from the fresh tissue indirectly using 2,3,5- Tiphenyl tetrazolium chloride, following the method of [12]. The values of treated samples were expressed as percent cellular respiration with respect to control.

E. Statistical analysis

For each treatment, five replicates were maintained and the entire experiment was repeated. The data were expressed as mean of the respective parameter and the significance (with respect to control) of the treatment was tested applying ANOVA and DMRT using the statistical package of SPSS version 10.

III. RESULTS AND DISCUSSION

There was a decrease in percent germination of *Amaranthus viridis* in response to different increasing concentrations of citronellol (Table 1). At 1 µL citronellol treatment, about 10% reduction in germination of the test weed was observed while a complete inhibition of germination was observed at a concentration of 2 µLcitronellol treatment. Further, growth in terms of radicle length of the test weed was considerably reduced compared to control. Radicle length was reduced by over 20% with the treatment of 0.1 µL while a reduction of over 70% was seen with 1 µL citronellol treatment (Table 1).

Table 1: Effect of citronellol on percent germination and radicle length (cm) of A. viridis.

Different superscripts in a column represent significant difference at $p < 0.05$.

Concentration (µL)	Percent Germination	Radicle length (cm)
Control	100 ± 0^{a}	3.3 ± 0.49^{a}
0.1	100 ± 0^{a}	2.56 ± 0.70 (77.58)^{b}
0.5	100 ± 0^{a}	2.21 ± 0.43 (66.97)^{c}
0.7	98.67 ± 1.15^{a}	1.5 ± 0.21 (45.45)^{d}
1	91.33 ± 2.31^{b}	0.91 ± 0.09 (27.58)^{e}
2	-	-

Table 2: Effect of citronellol on plumule length (cm) and seedling dry weight (mg) of A. viridis

Concentration (µL)	Plumule Length (cm)	Seedling dry weight (mg)
Control	1.9 ± 0.17^{a}	0.47 ± 0.12^{a}
0.1	1.87 ± 0.29 (98.42)^{a}	0.43 ± 0.07 (91.49)^{a}
0.5	1.21 ± 0.14 (63.68)^{b}	0.42 ± 0.12 (89.36)^{a}
0.7	1.2 ± 0.24 (63.16)^{b}	0.39 ± 0.08 (82.98)^{b}
1	0.74 ± 0.22 (38.95)^{c}	0.28 ± 0.05 (59.57)^{c}
2	-	-

Different superscripts in a column represent significant difference at $p < 0.05$.

The plumule length of the test weed was significantly inhibited with increasing concentrations of citronellol. With the treatment of 0.5 µL, plumule length was reduced by about 40% while an inhibition of about 60% was seen at a concentration of 1 µL citronellol (Table 2). Not only radicle and plumule lengths, but seedling dry weight was also affected negatively in *A. viridis* where it was reduced by about 10% compared to control with the treatment of 0.1 µL. The decrease was more in response to higher concentrations of citronellol. The seedling dry weight was reduced by around 40% with the treatment of 1 µL citronellol (Table 2). Although the exact mechanism by which the seedling growth of *A. viridis* is inhibited could not be ascertained from the present study, it could be associated with the inhibition of mitosis. [14] reported oils from *Cinnamomum zeylanicum* Blume and *Thymus vulgaris* L. to inhibit potato sprouts by killing their meristematic cells. Cineoles are reported to inhibit mitosis [3], [10], [15]. Thus, the inhibition of germination and early seedling growth of the weed *A. viridis* observed in the present study could be due to the disruption of mitotic activity.

Further, the chlorophyll content and percent cellular respiration in *A. viridis* seedlings were also drastically affected in response to citronellol. At the concentration of 0.1 µL, the content of chlorophyll was reduced by about 3% while at 0.5 µL, nearly 25% reduction was noticed. At 1 µL concentration, it was reduced by over 25% (Table 3). Whether the decrease in chlorophyll content was due to its reduced synthesis or enhanced degradation could not be investigated from the present study, yet this reduction in the chlorophyll content has a direct impact on the photosynthetic efficiency of the plants. [16] reported that both the reduction in the synthesis of the chlorophyll pigment and its enhanced degradation are responsible for the decrease in the overall content of chlorophyll in response to treatment with phenolic acids.

Table 3: Effect of citronellol on chlorophyll content (µg / mg) and % respiration of A. viridis

Concentration (µL)	Chlorophyll content (µg / mg)	Percent respiration
Control	8.66 ± 0.08^{a}	100 ± 1.65^{a}
0.1	8.39 ± 0.19 (96.88)^{b}	86.88 ± 0.42^{b}
0.5	6.56 ± 0.47 (75.75)^{c}	64.96 ± 0.66^{c}
0.7	6.44 ± 0.97 (74.36)^{cd}	55.32 ± 0.99^{d}
1	6.37 ± 0.62 (73.56)^{d}	41.63 ± 0.40^{e}
2	-	-

Different superscripts in a column represent significant difference at $p < 0.05$.

In addition to this, even the percent cellular respiration of the test weed *A. viridis* was considerably reduced in response to citronellol (Table 3). A significant reduction of about 35% was observed with the treatment of 0.5 µL and about 60% with the treatment of 1 µL. The reduction continued with each increasing concentration of the monoterpene. An inhibition in respiration shows that citronellol adversely affects the cellular energy production in *A. viridis*. This interference of the monoterpenes with the mitochondrial respiration is reported to be the cause of their adverse affects on the germination and growth of the plants [8]. Further, [1] reported that monoterpenes affect respiration by acting as uncouplers of oxidative phosphorylation.

IV. Conclusion

It is clear from the above study that citronellol exerts an overall inhibitory effect on the percent germination, seedling growth (in terms of radicle length, plumule length and seedling dry weight) and physiology of the weed *A. viridis.* Thus, it is concluded that it can be either used directly as a bio-herbicide or can act as a lead molecule for future weed management programmes.

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