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PHYTOTOXICITY OF LIMONENE AGAINST *AMARANTHUS VIRIDIS* L.

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ABSTRACT
A study was undertaken to assess the phytotoxic / allelopathic effect of limonene, a volatile monoterpene against the weed – *Amaranthus viridis* L. The germination of the weed was significantly inhibited even at very low concentrations (0.1 and 0.5 μL) of limonene where about 4 and 11% inhibition were observed respectively. Not only germination, but the seeding growth of the test weed in terms of radicle length, seedling length and seedling dry weight were appreciably reduced in response to limonene. At the concentration of 0.7 μL, the seedling length was reduced by about 25% while seedling dry weight was reduced by about 9%. Similarly, there was a significant reduction in the chlorophyll content and respiratory activity of the test weed which were reduced by over 50% upon treatment with 0.7 μL limonene. On the basis of this study, it is concluded that limonene has a weed suppressing potential and therefore, can be used for future weed management programmes either directly or by serving as a lead molecule.

INTRODUCTION

Huge amounts of synthetic herbicides have been extensively used for the control of weeds during the past few decades. 75% of the crop protection in the United States alone utilizes herbicides (Duke, 1999). However, these are being slowly replaced by safer and environment-friendly products owing to the health, environmental and toxicological problems associated with their use (Macias *et al*., 2001). In this direction, the use of natural plant products, which are environment-friendly and have different modes of action can serve as a good alternative to synthetic herbicides for weed management (Dayan *et al*., 2000). Among natural plant products, volatile monoterpenes are promising owing to their strong phytotoxicity (Singh *et al*., 2003). For example, ? and ? - pinene, limonene and citronellol extracted from the leaves of *Citrus aurantium* L. inhibited the growth of the weed *Amaranthus retroflexus* L. (Al Saadawi *et al*., 1985). A reduction in the growth of grasses has also been reported due to the production of volatile terpenes produced by *Salvia* sp. (Muller and Muller, 1964). Limonene is one such monoterpene, which is a major component of essential oils from a number of aromatic species like *Syzygium aromaticum* (Linn.) Merill and Perry. However, very little has been done to determine its allelopathic ability against weedy species. In the present investigation, the phytotoxic effect of limonene against the weedy species – *Amaranthus viridis* was investigated. The objective of the present study is to explore the herbicidal potential of limonene for the management of obnoxious weeds.

MATERIALS AND METHODS

Collection of material

Seeds of hairy beggar's tick (*Amaranthus viridis* L.) were collected from locally growing wild stands in the campus of Panjab University. Limonene of technical grade was procured from Lancaster, U.K.

Bioassay studies

Fifty seeds of hairy beggar's tick 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 limonene was given in concentrations ranging from 0.1, 0.5, .0.7, 1, 2, 5 and 7 μL and Petri dishes were properly sealed with a cellotape. A similar set up without limonene treatment 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 $\pm$ 2ºC and 75 $\pm$ 2 % relative humidity with photoperiod of 16 / 8 h day / night. After 7 days, number of seeds that germinated was counted and redicle length, seedling length and dry weight were measured.

Estimation of chlorophyll content

Chlorophyll was extracted from 25 mg of cotyledonary leaves of *A. viridis* (treated with different concentrations of limonene) in 4 mL of dimethyl sulphoxide by the method of Hiscox and Israelstam (1979). Chlorophyll content was determined spectrophotometrically using the equation of Arnon (1949) and expressed in terms of dry weight of the tissue as per Rani

and Kohli (1991).

Determination of cellular respiration

The cellular respiration was determined from the fresh tissue indirectly using 2,3,5- triphenyl tetrazolium chloride, following the method of Steponkus and Lanphear (1967). The values of treated samples were expressed as precent cellular respiration with respect to control.

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 of treatment was tested with respect to control applying ANOVA and DMRT using the statistical package of SPSS version 10.

RESULTS AND DISCUSSION

In response to different concentrations of limonene, there was a decrease in germination of the test weed. A complete inhibition in germination was achieved at a concentration of 7 μL. Further, growth in terms of radicle length and seedling length of the test weed was considerably reduced compared to control. Radicle length was reduced by about 85% with the treatment of 2 μL while a reduction of about 90% was seen with 5 μL limonene treatment (Table 1).

Table 1: Effect of limonene on percent germination and radicle length of *A. viridis*

Concentration (μL)	Percent Germination	Radicle length (cm)
Control	100 ± 1.25[a]	3.51 ± 0.86[a]
0.1	96.46 ± 1.48[a]	3.36 ± 0.09 (95.73)[a]
0.5	88.66 ± 2.35[b]	3.19 ± 0.08 (90.88)[b]
0.7	86.36 ± 1.93[b]	2.97 ± 1.16 (84.62)[c]
1	44.31 ± 5.77[c]	1.39 ± 0.43 (39.60)[d]
2	19.32 ± 1.92[d]	0.54 ± 0.26 (15.38)[e]
5	18.18 ± 1.92[d]	0.34 ± 0.14 (9.69)[e]
7	0[e]	-

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

Seedling length also decreased with increasing concentrations of limonene. With the treatment of 1 μL, seedling length was reduced by about 70% while an inhibition of about 90% was seen at a concentration of 5 μL limonene (Table 2). Similarly, seedling dry weight was also affected in *A. viridis* where it was reduced by about 17% compared to control with the treatment of 1 μL while it was reduced by around 33% with the treatment of 5 μL limonene (Table 2). Although the exact mechanism by which the growth is inhibited could not be determined from the present study, it could be associated with the inhibition of mitosis. Cineoles are already known to inhibit mitosis (Vaughn and Spencer, 1993; Baum *et al*., 1998 and Romagni *et al*., 2000). Thus, disruption of mitotic activity may be responsible for observed inhibition of germination and growth of the weed *A. viridis* in the present study.

Not only germination and earlier growth, even the chlorophyll content and cellular respiration were also drastically affected in *A. viridis* seedlings in response to limonene. At the lowest concentration of 0.1 μL, nearly 20% reduction was observed in the content of chlorophyll. The decrease was more in response to higher concentrations of limonene and at 1 μL concentration, it was reduced by about 80% (Table 3). Whether the decrease in chlorophyll content was due to its reduced synthesis or enhanced degradation could not be ascertained from the present study, yet this reduction in the chlorophyll content has a direct impact on the photosynthetic efficiency of the plants. Yang *et al*., (2004) reported that both the reduction in synthesis of the chlorophyll pigment as well as the increase in its degradation are responsible for the decrease in the overall content of chlorophyll in response to phenolic acids treatment.

Table 2: Effect of limonene on seedling length and seedling dry weight of *A. viridis*.

Concentration (μL)	Seedling Length (cm)	Seedling dry weight (mg)
Control	7.26 ± 1.09[a]	0.69 ± 0.13[a]
0.1	6.77 ± 0.46 (93.25)[b]	0.67 ± 0.12 (97.10)[a]
0.5	6.15 ± 0.39 (84.71)[c]	0.63 ± 0.07 (96.88)[a]
0.7	5.44 ± 1.26 (74.93)[d]	0.62 ± 0.11 (91.30)[b]
1	2.13 ± 0.48 (29.34)[e]	0.53 ± 0.04 (82.81)[c]
2	1.35 ± 0.30 (18.60)[f]	0.44 ± 0.02 (68.75)[d]
5	0.82 ± 0.25 (11.29)[g]	0.43 ± 0.06 (67.19)[d]
7	-	-

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

Not only the chlorophyll content, even the cellular respiration of the test weed, *A.viridis* was reduced in response to limonene (Table 3). A reduction of about 50% was observed with the treatment of 0.5 μL and the reduction continued with higher treatments of the monoterpene. Such an inhibitory effect on respiration indicates that limonene adversely affects the cellular energy production in *A. viridis*. Penuelas *et al*., (1996) reported that the interference of the monoterpenes with the mitochondrial respiration is responsible for their adverse affects on the germination and growth of the plants.

Table 3: Effect of limonene on chlorophyll content and percent respiration of *A. viridis*

Concentration (μL)	Chlorophyll content (μg / mg)	Percent respiration
Control	15.5 ± 0.07[a]	100 ± 1.0[a]
0.1	12.34 ± 0.47 (79.61)[b]	72.34 ± 0.48[b]
0.5	9.85 ± 0.36 (63.55)[c]	53.78 ± 0.06[c]
0.7	7.32 ± 0.32 (47.23)[d]	45.60 ± 1.62[d]
1	3.47 ± 0.42 (22.39)[e]	36.06 ± 1.24[e]
2	2.41 ± 0.05 (15.55)[f]	28.97 ± 0.41[f]
5	0.68 ± 0.08 (4.4)[g]	13.18 ± 4.26[g]
7	-	-

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

CONCLUSION

It is clear from the present study that limonene exerts an overall inhibitory effect on the germination, growth and physiology of the weed *A. viridis* and thus, can serve as a good candidate for the synthesis of bioherbicides for future weed management programmes.

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