International Journal of Applied Science and Mathematics
Volume 2, Issue 6, ISSN (Online): 2394-2894

Inhibition Potential of Limonene, A Volatile Monoterpene, Towards *Bidens pilosa* L.

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Abstract **– A study was undertaken to assess the phytotoxic effect of limonene, a volatile monoterpene towards the weed – *Bidens pilosa* L. The germination of the weed was significantly inhibited even at low concentrations (25 and 50 µL) of limonene where about 6 and 9% inhibition were observed, respectively. Not only germination, but the seedling 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 50 µL, the seedling length was reduced by over 50% while seedling dry weight was reduced by about 20%. Similarly, there was a significant reduction in the chlorophyll content and respiratory activity of the test weed upon treatment with various increasing concentrations of limonene. These were reduced by about 65% and 80%, respectively upon treatment with 100 µL limonene. On the basis of this study, it is concluded that limonene has a weed suppressing ability and therefore, can be used for future weed management programmes either directly or by serving as a lead molecule.**

Keywords **– *Bidens pilosa*, Limonene, Phytotoxicity, Volatile Monoterpene, Weed.**

I. INTRODUCTION

Synthetic herbicides have been extensively used for the control of weeds during the past few decades. In the United States alone, 75% of the crop protection utilizes herbicides [6]. However, owing to the health, environmental and toxicological problems associated with their use, these are being slowly replaced by safer and environment-friendly products [11]. 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 [4]. Among natural plant products, volatile monoterpenes are promising owing to their strong phytotoxicity [18]. For example, α and β - pinene, limonene and citronellol extracted from the leaves of *Citrus aurantium* L. inhibited the growth of the weed *Amaranthus retroflexus* L. [1]. A reduction in the growth of grasses has also been reported due to the production of volatile terpenes produced by *Salvia* sp. [13]. 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 – *Bidens pilosa* L. was investigated. Thus, the objective of the present study is to explore the possible potential of limonene as a natural and environment-friendly herbicide for the management and control of obnoxious weeds.

II. MATERIALS AND METHODS

A. Collection of material

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

B. 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 25, 50, 100, 150, 200, 225, 250 and 300 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 ± 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, seedling length and dry weight were measured.

C. Estimation of chlorophyll content

Chlorophyll was extracted from 25 mg of cotyledonary leaves of *B. pilosa* (treated with different concentrations of limonene) in 4 ml of dimethyI sulphoxide by the method of [8]. Chlorophyll content was determined spectro-photometrically using the equation of [2] and expressed in terms of dry weight of the tissue as per [15].

D. 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 [19]. 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 of treatment was tested with respect to control applying ANOVA and DMRT using the statistical package of SPSS version 10.

III. RESULTS AND DISCUSSION

In response to different concentrations of limonene, there was a decrease in germination of the test weed (Table 1). A complete inhibition in germination was achieved at a concentration of 300 µL. Further, growth in terms of radicle length, seedling length and seedling dry weight of the test weed was considerably reduced

compared to control. Radicle length was reduced by about 50% with the treatment of 100 µL while a reduction of about 70% was seen with 200 µL limonene treatment (Table 1). The decrease in germination has also been reported in response to essential oils of a number of aromatic plants [5], [9], [12], [13], [17] and [22].

A decrease in terms of the seedling length of the test weed was also noticed with increasing concentrations of limonene. With the treatment of 25 µL, seedling length was reduced by about 40% while an inhibition of about 80% was seen at a concentration of 200 µL limonene (Table 2). Similarly, seedling dry weight was also affected in *B.pilosa* where it was reduced by about 50% compared to control with the treatment of 200 µL while it was reduced by around 80% with the treatment of 250 µL limonene (Table 2). The exact mechanism by which the growth is inhibited could not be determined from the present study. However, [20] reported that essential oils obtained from cinnamon (*Cinnamomum zeylanicum* Blume) and red thyme (*Thymus vulgaris* L.) inhibit potato sprouts by killing their meristematic cells. Earlier, [10] had reported disruption of mitotic activity and reduction in root tip development in onion by volatile oils from purple sage (*Salvia leucophylla* Greene).

A decrease in terms of the seedling length of the test weed was also noticed with each increasing concentration of limonene. With the treatment of 25 µL, seedling length was reduced by about 40% while an inhibition of about 80% was seen at a concentration of 200 µL limonene (Table 2). Similarly, seedling dry weight was also affected in *B. pilosa* where it was reduced by about 50% compared to control with the treatment of 200 µL while it was reduced by around 80% with the treatment of 250 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.

Table 1: Effect of limonene on percent germination and radicle length of B. pilosa.

Concentration (µL)	Percent Germination	Radicle length (cm)
Control	100 ± 0^{a}	3.38 ± 0.46^{a}
25	94.43 ± 0.58^{b}	$2.3 \pm 0.43\ (68.05)^{b}$
50	91.10 ± 0.58^{b}	$1.99 \pm 0.36\ (58.88)^{c}$
100	88.90 ± 1.15^{c}	$1.67 \pm 0.16\ (49.41)^{d}$
150	85.57 ± 0.58^{c}	$1.28 \pm 0.12\ (37.87)^{e}$
200	67.77 ± 0.58^{d}	$1.03 \pm 0.06\ (30.47)^{f}$
225	49.95 ± 0.48^{e}	$0.97 \pm 0.14\ (28.70)^{f}$
250	10.02 ± 0.16^{f}	$0.19 \pm 0.01\ (5.62)^{g}$
300	0^{g}	-

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

Cineoles are already known to inhibit mitosis [21], [3] and [16]. Thus, disruption of mitotic activity may be responsible for observed inhibition of germination and growth of the weed *B. pilosa* in the present study.

Not only germination and earlier growth, even the chlorophyll content and cellular respiration were also drastically affected in *B. pilosa* seedlings in response to limonene. At the lowest concentration of 25 µL, nearly 46% reduction was observed in the content of chlorophyll. The decrease was more in response to higher concentrations of limonene and at 200 µL concentration, it was reduced by about 90% (Table 3). Whether the decrease in chlorophyll content was due to its reduced synthesis or enhanced degradation, yet this reduction in the chlorophyll content has a direct impact on the photosynthetic efficiency of the plants. [23] 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 B.pilosa

Concentration (µL)	Seedling Length (cm)	Seedling dry weight (mg)
Control	7.06 ± 1.15^{a}	0.52 ± 0.08^{a}
25	$4.11 \pm 0.33\ (58.22)^{b}$	$0.47 \pm 0.13\ (90.38)^{b}$
50	$3.36 \pm 0.35\ (47.59)^{bc}$	$0.43 \pm 0.04\ (82.69)^{c}$
100	$2.58 \pm 0.14\ (36.54)^{c}$	$0.42 \pm 0.02\ (80.77)^{c}$
150	$1.80 \pm 0.29\ (25.50)^{d}$	$0.39 \pm 0.06\ (75.0)^{d}$
200	$1.49 \pm 0.14\ (21.10)^{d}$	$0.25 \pm 0.07\ (48.08)^{e}$
225	$1.18 \pm 0.11\ (16.71)^{d}$	$0.24 \pm 0.06\ (46.15)^{e}$
250	$0.23 \pm 0.05\ (3.26)^{e}$	$0.09 \pm 0.01\ (17.31)^{f}$
300	-	-

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

Not only the chlorophyll content, even the percent cellular respiration of the test weed, *B. pilosa* was considerably reduced in response to limonene (Table 3). A significant reduction of about 70% was observed with the treatment of 25 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 *B. pilosa*. The interference of the monoterpenes with the mitochondrial respiration is reported to be responsible for their adverse affects on the germination and growth of the plants [13].

Table 3: Effect of limonene on chlorophyll content and percent respiration of B. pilosa

Concentration (µL)	Seedling Length (cm)	Seedling dry weight (mg)
Control	7.06 ± 1.15^{a}	0.52 ± 0.08^{a}
25	$4.11 \pm 0.33\ (58.22)^{b}$	$0.47 \pm 0.13\ (90.38)^{b}$
50	$3.36 \pm 0.35\ (47.59)^{bc}$	$0.43 \pm 0.04\ (82.69)^{c}$
100	$2.58 \pm 0.14\ (36.54)^{c}$	$0.42 \pm 0.02\ (80.77)^{c}$
150	$1.80 \pm 0.29\ (25.50)^{d}$	$0.39 \pm 0.06\ (75.0)^{d}$
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250	$0.23 \pm 0.05\ (3.26)^{e}$	$0.09 \pm 0.01\ (17.31)^{f}$
300	-	-

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

IV. CONCLUSION

The present study clearly shows that limonene exerts an overall inhibitory effect on the germination, seedling growth and physiology of the weed *B. pilosa* and thus, can serve as a good candidate for the synthesis of bioherbicides for future weed management programmes.

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