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Potential of Linalool for Inhibition of *Cassia occidentalis*

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A B S T R A C T

A study was carried out to assess the inhibitory potential of linalool, a volatile monoterpene found in many flowers and spice plants against coffee-weed, *Cassia occidentalis*. Linalool was found to have a significant inhibitory effect on the germination and early seedling growth of the test weed. The germination in the test weed was reduced by about 17% at the lowest concentration of 0.2 nl/cc and a complete germination inhibition was observed at 20 nl/cc linalool treatment. Similarly, the seedling growth of the test weed in terms of radicle length, seedling length and seedling dry weight was appreciably reduced in response to various increasing concentrations of linalool. Likewise, the physiological parameters *viz.* the content of total chlorophyll and percent cellular respiration of the test weed were also reduced to varying degrees compared to control, indicating a negative effect of the test monoterpene on the photosynthetic efficiency and energy metabolism of the weed species. Based on the above study, it is concluded that linalool possesses weed-suppressing abilities and can be employed for the management of obnoxious weeds.

Introduction

Volatile monoterpenes, the constituents of essential oils, are the simplest chemical molecules belonging to the terpenoid family and possess multiple biological and ecological functions. Because of their natural aroma, they find extensive use in food, perfumery, cosmetic industries and aromatherapy. They also play many important roles including plant-plant interactions, defense mechanism as plant protectants against herbivory and as pollinator-attractants and thus help in maintaining the delicate balance of ecosystems (Swain, 1977; Fischer, 1991; Vokou, 1999; Paiva, 2000). As potent inhibitors of seed germination, these monoterpenes are involved in allelopathic interactions among plants and thus play an important role in maintaining, structuring and patterning of plant communities in natural ecosystems (Muller, 1965; Asplund, 1968; Abrahim *et al.*, 2000; Singh *et al.*, 2002a, b; Weidenhamer *et al.*, 1993; Vokou, 1999). Besides this, they are easily biodegradable and possess little toxicity against mammals and other non-target species. These properties make these chemicals immensely suitable for agro-industry (Isman, 2000; Beuchat, 2001). Some studies have already reported their use as potential chemicals for the management of weeds and pests in sustainable agriculture (Isman, 2000; Romagni *et al.*, 2000; Singh *et al.*, 2002a, b).

Various monoterpenes like citronellal, citronellol and cineole have been observed to inhibit germination and initial seedling growth of several weeds such as *Amaranthus viridis*, *Echinochloa crus-galli* and *Bidens pilosa* under *in vitro* conditions (Singh *et al.*, 2002b, 2004). Linalool is a monoterpene present in a number of flower and spice plants like *Lavandula officinalis, Thymus vulgaris, Ocimum basilicum, Cinnamomum tamala, Artemisia vulgaris* and *Cannabis sativa*. Inspite of abundant availability and high phytotoxicity, the potential of linalool as a bio-herbicide still remains to be investigated.
The present investigation was therefore undertaken to explore the phytotoxic effect of linalool against a weedy species, *Cassia occidentalis* with a view to explore its herbicidal potential.

Materials and Methods

Collection of Materials

Seeds of Coffee weed (*Cassia occidentalis* L.) were collected locally from wildly growing stands in the campus of Panjab University, Chandigarh. Linalool was purchased from Lancaster, UK. The seeds were surface sterilized.

Bioassay Studies

Seeds of *Cassia occidentalis* were divided into 9 groups of 50 each and dipped in distilled water for 16 h for imbibition prior to germination trials. These were then equidistantly placed in 6” diameter Petri dishes lined with two layers of moistened Whatman no. 1 filter paper. The treatment of linalool was given in concentrations ranging from 0.2, 1, 1.5, 2, 5, 10, 15 and 20 nl/cc volume of Petri dishes. After the addition of the volatile monoterpene, the Petri dishes were properly sealed. A similar set-up without the treatment of linalool served as control. For each treatment, 5 replicates were maintained. The entire set up was kept in an environmentally controlled seed germinating chamber at 25 ± 2 °C and 75 ± 2 % relative humidity with a photoperiod of 16/8 day/night. After a week, the number of seeds that germinated was counted, radicle length, seedling length and seedling dry weight were measured and the total chlorophyll content and percent respiratory activity were determined.

Estimations

Chlorophyll was extracted from 25 mg of tissue in 4 ml of Dimethyl sulphoxide (DMSO) following Hiscox and Israelstam (1979). The extinction values of chlorophyll thus recovered in DMSO were 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 (Arnon, 1949) and expressed on dry weight basis as per Rani and Kohli (1991). Values on dry weight equivalents were calculated by placing same amount of tissue in an oven at 80^{o}C for 24 hr.

Respiratory values were determined from the fresh plant tissue indirectly using 2,3,5-

triphenyl tetrazolium chloride following the method of Steponkus and Lanphear (1967). This is an indirect measure of cellular respiration whereby formation of red coloured formazan occurs due to trapping of the oxygen molecules released through the respiratory chain. The values of treated samples thus obtained were expressed as percent cellular respiration with respect to control.

Statistical Analysis

The data of percent germination, radicle length, seedling length, seedling dry weight, chlorophyll content and respiratory activity was analyzed by one-way ANOVA followed by Duncan's multiple range test.

Results and Discussion

It is very clear from the results that in response to linalool, germination of *Cassia occidentalis* was reduced to varying degrees depending upon the concentration of the former (Table 1). At the lowest concentration of 0.2 nl/cc linalool treatment, germination was reduced by about 17% while at 15 nl/cc, a reduction of over 60% was observed. A complete inhibition of germination of the test weed was observed at a concentration of 20 nl/cc. Similarly, the radicle length of the test weed was also significantly reduced in response to the monoterpene. At 1 nl/cc treatment, radicle length was reduced to about 83% while it was reduced to 41% at 15 nl/cc compared to control (Table 1).

Further, seedling growth measured in terms of seedling length and seedling dry weight of the test weed was also significantly reduced compared to control (Table 2). At 0.2 nl/cc treatment, seedling length was reduced by 11% while it was reduced by about 63% at 15 nl/cc compared to control. At the concentration of 1 nl/cc linalool treatment, seedling dry weight was reduced by about 17% while at 15 nl/cc, a reduction of about 45% was observed. The observed inhibition of germination and initial growth of the weedy species could be due to the disruption of mitotic activity in the germinating seeds.

This is strengthened by references available in literature (Romagni *et al*., 2000). Several reasons have been put forward to determine the factors that disrupt mitosis including disruption of microtubule organization or alternation of cell wall biosynthesis (Lehnen and Vaughn, 1992).

Likewise, the content of chlorophyll was significantly less in linalool-treated samples compared to control. At the lowest concentration of 0.2 nl/cc, chlorophyll content was reduced by about 23% while at 15 nl/cc, a reduction of around 62% was observed (Table 3). The decrease in chlorophyll content points towards the diminishing photosynthetic efficiency in response to the test monoterpene. The mechanism behind the decrease in chlorophyll content in the target weed (whether it is due to its decreased synthesis or enhanced degradation) could not be ascertained from the present study. However, references available in literature indicate reduction in the levels of chlorophyll pigment in response to allelochemicals (Romagni *et al*., 2000; Singh *et al*., 2002b).

An appreciable reduction in cellular respiration was also observed in *C. occidentalis* when treated with linalool (Table 3). At the lowest concentration of 0.2 nl/cc, cellular respiration was reduced by about 13% while at 15 nl/cc, a reduction of around 85% was observed.

Table.1 Effect of Linalool on the Percent Germination and Radicle Length (cm) of *C. occidentalis*

Concentration (nl/cc)	Percent Germination	Radicle Length (cm)
0	**100** ± 0[a]	**4.62** ± 0.2[a]
0.2	**83.33** ± 11.55[b]	**4.18** ± 0.31 (90.48)[b]
1	**73.33** ± 10.23[c]	**3.83** ± 0.09 (82.90)[c]
1.5	**64.21** ± 10.08[d]	**3.42** ± 0.11(74.03)[d]
2	**56.67** ± 5.77[e]	**3.09** ± 0.03 (66.88)[d]
5	**46.56** ± 4.78[f]	**2.53** ± 0.04 (54.76)[e]
10	**43.33** ± 6.55[f]	**2.16** ± 0.06 (46.75)[f]
15	**36.67** ± 5.85[g]	**1.9** ± 0.09 (41.13)[g]
20	**-**	**-**

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

Table.2 Effect of Linalool on the Seedling Length (cm) and Seedling Dry Weight (mg) of *C. occidentalis*

Concentration (nl/cc)	Seedling Length (cm)	Seedling Dry Weight (mg)
0	**11.7** ± 0.27[a]	**11.39** ± 0.08
0.2	**10.40** ± 0.31 (88.89)[b]	**10.92** ± 0.18 (95.87)[b]
1	**9.5** ± 0.12 (81.20)[c]	**9.5** ± 0.12 (83.41)[c]
1.5	**8.81** ± 0.07 (75.30)[d]	**9.28** ± 0.35 (81.47)[c]
2	**8.11** ± 0.05 (69.32)[e]	**8.22** ± 1.23 (72.17)[d]
5	**7.16** ± 0.35 (61.20)[f]	**7.28** ± 0.16 (63.92)[e]
10	**6.28** ± 0.11 (53.68)[g]	**6.92** ± 0.05 (60.76)[f]
15	**4.32** ± 0.34 (36.92)[h]	**6.3** ± 0.24 (55.31)[g]
20	**-**	**-**

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

Table.3 Effect of Linalool on the Total Chlorophyll Content (µg/mg) and Percent Cellular Respiration of *C. occidentalis*

Concentration (nl/cc)	Total Chlorophyll Content (µg/mg)	Percent Cellular Respiration
0	**8.72** ± 0.13[a]	**100** ± 1.23[a]
0.2	**6.75** ± 0.04 (77.41)[b]	**86.70** ± 1.9[b]
1	**6.59** ± 0.04 (75.57)[b]	**71.36** ± 1.54[c]
1.5	**6.53** ± 0.05 (74.89)[b]	**49.80** ± 0.76[d]
2	**6.17** ± 0.08 (70.76)[c]	**46.21** ± 1.01[d]
5	**5.43** ± 0.09 (62.27)[d]	**40.67** ± 1.54[e]
10	**3.80** ± 0.07 (43.58)[e]	**24.50** ± 0.76[f]
15	**3.32** ± 0.02 (38.03)[f]	**15.79** ± 1.25[g]
20	**-**	**-**

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

Conclusion

It is clear from the present study that linalool has a potential to reduce the germination, early growth and development of the weed species and thus could prove very useful for future weed management programmes either directly or by serving as a lead molecule.

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