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PHYTOTOXIC EFFECT OF EUGENOL TOWARDS TWO WEEDY SPECIES

S. VAID*, DAIZY R. BATISH, H. P. SINGH[1] AND R. K. KOHLI
Department of Botany, Panjab University, Chandigarh - 160 014
[1]Department of Environment and Vocational Studies,
Panjab University, Chandigarh - 160 014
E-mail: dr.supriyavaid@yahoo.com

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***Corresponding author**

ABSTRACT
A study was undertaken to assess the inhibitory / allelopathic potential of eugenol–a volatile monoterpene found in *Eugenia* spp. and several other aromatic plants, against two weedy species *viz*. *Cassia occidentalis* and *Bidens pilosa*. Eugenol significantly inhibited the germination of both the weeds even at very low concentration. However, the effect was more on *B. pilosa* compared to *C. occidentalis*. Likewise, the seedling growth measured in terms of seedling length and dry weight of both the test weeds was appreciably reduced in response to the test monoterpene. Not only the growth, even the content of the total chlorophyll and cellular respiration in both the test weeds were appreciably reduced, thereby indicating that eugenol negatively affects the photosynthetic efficiency and the energy metabolism of the weed species. Based on the study, it is concluded that eugenol possesses weed-suppressing ability and can be used for future weed management programmes either directly or by serving as a lead molecule.

INTRODUCTION

Weeds–the essential component of agroecosystems, interfere with crops and lead to enormous crop losses. Indiscriminate use of synthetic herbicides though has undoubtedly enhanced the much needed crop production, yet have led to a number of toxicological and environmental problems causing ill effects on human health (Macias *et al*., 2001). Therefore, efforts are being made world over to search for safer and environmentally benign chemicals that have relatively shorter half-life. In this direction, biologically active natural products such as allelochemicals are fast being tested for weed management since they are environment friendly and have different modes of action (Dayan *et al*., 2000). Besides, they also exhibit immense diversity and biological activity.

Among the natural plant products, volatile terpenes have been shown to be promising with potential weed suppressing ability (Kohli *et al*., 1998; Singh *et al*., 2002). These are commonly found as components of essential oils in a number of aromatic plants, e.g. *Salvia* spp, *Eucalyptus* spp., *Artemisia* spp. and *Pinus* spp. Besides, they also exhibit considerable phytotoxicity, e.g. volatile terpenes of *Salvia leucophylla* are most effective in inhibiting the growth of grasses (Muller *et al*., 1964); *Eucalyptus* volatile terpenes reduce growth of a number of plants (Kohli and Singh, 1991); cineoles (1, 4- and 1, 8-) reduce the growth of weeds (Romagni *et al*., 2000). Eugenol is one such monoterpene, which is a major component of essential oils from a number of aromatic species including *Eugenia* spp. and is biologically very active (Tworkoski, 2002). But very little has been done to determine its allelopathic activity against weedy species. With a view to explore its herbicidal potential against a wide range of weeds, the present investigation was, therefore, undertaken to explore the phytotoxic effect of eugenol against two weedy species *viz*. *Cassia occidentalis* and *Bidens pilosa*. The objective of this study, therefore, is to explore the possible utility of eugenol for the management of obnoxious weeds and to determine its effect on physiological changes in plants.

MATERIALS AND METHODS

Collection of Material

Seeds of coffee weed (*Cassia occidentalis* L.) and hairy beggarticks (*Bidens pilosa* L.) were collected locally from wildly growing stands in the campus of Panjab University, Chandigarh. These were surface sterilized. Eugenol was purchased from Lancaster, UK.

Bioassay Studies

Seeds of both the weed species were divided into 9 groups of 50 each and dipped in distilled water for 16 hrs for imbibition prior to germination trial. These were then equidistantly placed in 6" diameter Petri dishes lined with two layers of moistened Whatman no. 1 filter paper. The filter paper was treated with 1, 2, 5, 10 and 20 μL of eugenol per Petri dish. After the addition of the volatile monoterpenes, the Petri dishes were sealed. A similar set-up but without eugenol 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 hour day / night. After a week, seedling length and seedling dry weight were measured and the total chlorophyll content and 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). Its concentration was determined spectrophotometrically (Arnon, 1949) and the amount was expressed in terms of dry weight as suggested by Rani and Kohli (1991). Respiratory values were determined indirectly using 2, 3, 5 - triphenyl tetrazolium chloride as per the method of Steponkus and Lanphear (1967).

Statistical analysis

The data of percent germination, seedling growth, chlorophyll content and respiratory activity were analyzed by one-way ANOVA followed by Duncan's multiple range test.

RESULTS AND DISCUSSION

It is clear from the results that in response to different concentrations of eugenol, germination of both the weedy species was reduced (Fig. 1). Reduction in germination was more in case of *B. pilosa* compared to *C. occidentalis*. A complete inhibition of germination of *B. pilosa* was observed at 5μL concentration whereas in case of *C. occidentalis* it was seen at 20 μL (Fig. 1).

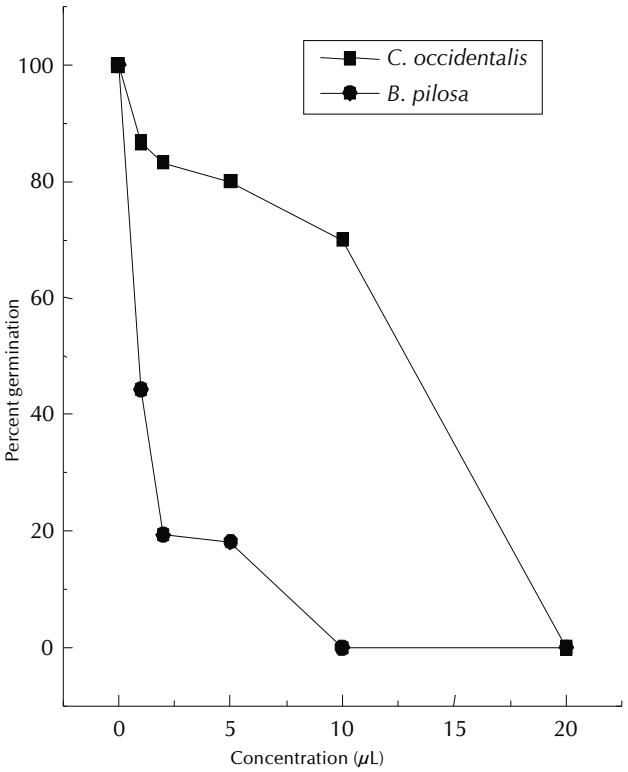

Figure 1: Effect of Eugenol on the germination of two test weed species

Further, seedling length and seedling dry weight of both the weeds were significantly reduced compared to control. Growth of *B. pilosa* was more affected than *C. occidentalis* (Table 1). Inhibition of germination and growth of both the weedy species could be due to disruption of mitotic activity in the germinating seeds. Though, present study does not have supporting data in this direction, yet references available in literature can strengthen this fact (Romagni *et al.*, 2000). Several reasons have been put forward to find out the factors that disrupt mitosis such as disruption of microtubule organization or alternation of cell wall biosynthesis (Lehnen and Vaughn, 1992).

The content of chlorophyll extracted in DMSO was significantly less in treated weed species compared to control. More reduction was observed in *B. pilosa* (Table 2). The decrease in chlorophyll suggests the diminishing photosynthesis efficiency in response to volatile terpenes. The mechanism as to how chlorophyll decreases in the target weed or whether it is due to decreased synthesis or enhanced

Table 1: Effect of Eugenol on the seedling length and seedling dry weight of *C. occidentalis* and *B. pilosa*

Concentration (μL)	Seedling length (cm)		Seedling Weight (mg)	
	C. occidentalis	*B. pilosa*	*C. occidentalis*	*B. pilosa*
0	9.33 ± 0.75^{a}	7.26 ± 1.09^{a}	10.4 ± 0.50^{a}	0.64 ± 0.05^{a}
1	6.38 ± 0.43^{b}	2.13 ± 0.48^{b}	7.70 ± 0.34^{b}	0.53 ± 0.05^{b}
2	5.83 ± 0.79^{c}	1.35 ± 0.30^{c}	7.13 ± 0.29b^{c}	0.49 ± 0.07^{bc}
5	3.94 ± 0.60^{d}	0.82 ± 0.25^{d}	6.73 ± 0.48^{c}	0.46 ± 0.03^{c}
10	2.06 ± 0.29^{e}	-	4.07 ± 0.37^{d}	-
20	-	-	-	-

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

Table 2: Effect of Eugenol on the total chlorophyll content and cellular respiration of *C. occidentalis* and *B. pilosa*

Concentration (μL)	Total Chlorophyll content (μg/mg)		Cellular Respiration (%)	
	C. occidentalis	*B. pilosa*	*C. occidentalis*	*B. pilosa*
0	8.75 ± 0.38^{a}	6.45 ± 0.07^{a}	100^{a}	100^{a}
1	4.40 ± 0.15^{b}	5.80 ± 0.42^{b}	47.3^{b}	36.1^{b}
2	3.96 ± 0.27^{c}	4.03 ± 0.05^{c}	45.3^{b}	29.0^{c}
5	3.40 ± 0.04^{d}	1.14 ± 0.8^{d}	40.4^{c}	13.2^{d}
10	0.16 ± 0.09^{e}	-	24.5^{d}	-
20	-	-	-	-

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

degradation could not be known. Nevertheless, there are available references indicating reduced levels of chlorophyll pigment in response to allelopathy / allelochemicals (Romagni *et al*., 2000; Singh *et al*., 2002).

The study on cellular respiration is an indirect method indicating rate at which O_2 molecule released through respiratory chain are trapped by 2, 3, 5 - triphenyl tetrazolium chloride (TTC) leading to the formation of water insoluble red formazan (Steponkus and Lanphear, 1967). The intensity of this red coloured formazan extracted in alcohol is determined. The present study reveals a considerable and appreciable reduction in cellular respiration in both the weed species when treated with eugenol (Table 2).

CONCLUSION

From the present study, it is clear that eugenol has a potential to reduce the growth and development of weed species and thus could be useful for future weed management programmes either directly or by serving as a the lead molecule.

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