International Journal of Advanced Technology in Engineering and Science
Vol. No.5, Issue No. 01, January 2017
www.ijates.com
ijates
ISSN 2348 - 7550

COMPARITIVE PHYTOTOXICITY OF THREE MONOTERPENES AGAINST *CASSIA OCCIDENTALIS*

Saroj Kumari Fagodia[1], Daizy Rani Batish[2], Harminder Pal Singh[3]

[1,2]*Department of Botany, Panjab University, Chandigarh 160014, (India)*

[3]*Department of Environment Studies, Panjab University, Chandigarh, (India)*

ABSTRACT

Monoterpenes, the chemical constituents of essential oils found in plants, are known biologically active compounds. The present study was carried out to investigate the phytotoxic potential of three monoterpenes - citronellol, β-pinene and α-terpinene against coffee-weed, Cassia occidentalis under laboratory conditions in order to assess their herbicidal activity. At tested concentrations (0.5, 1 .0 and 2.5 mM) all three monoterpenes showed varying response. Citronellol and β-pinene significantly inhibit % germination at 2.5 mM. Further, the growth of seedlings in terms of root length, soot length and seedling dry weight was appreciably reduced in response to citronellol and β-pinene. A reduction in chlorophyll content of the cotyledonary leaves of C. occidentalis was also noticed, indicating an adverse effect on photosynthesis. On the basis of overall phytotoxicity, potency of monoterpenes was in the order of citronellol > β-pinene > α-terpinene. The results from this study suggest that citronellol possess strongest phytotoxic potential and can thus serve as lead molecule for the synthesis of bioherbicides.

Key words: Bioherbicide, Chlorophyll content, Citronellol, Monoterpene, Phytotoxic

I. INTRODUCTION

In recent years, there has been an increase of public concern about the use of chemical products in agriculture and livestock. Also, there is an increasing social pressure for the replacement of these compounds by natural products. In the case of agriculture, an excessive use of pesticides and herbicides generates environmental pollution and toxic wastes which are harmful for human health [1]. The potential use of naturally derived products as new, effective, reduced-risk alternatives for weed management is a current topic. So, worldwide efforts are being made to search new eco-friendly chemicals as a source of natural herbicides. Plants are a virtually in exhaustible source of biologically active compounds with great structural diversity. Among several classes of natural plant products volatile monoterpenes have received much attention as strong inhibitors of plant growth, particularly in allelopathic studies [2, 3]. 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 [4, 5]. Their Phytotoxic nature towards weed species is being explored so as to use them for weed management purposes [6]. Moreover, they often have modes of action different from synthetic herbicides and may serve as prototype for the synthesis of lead molecules for further herbicides [7].

Thus studies on the relative phytotoxicity of monoterpenes should be undertaken to choose the best lead compounds. Keeping this in mind, we selected three monoterpenes *viz.* citronellol, α-terpinene, β-pinene to find out their comparative phytotoxicity towards *Cassia occidentalis* L. - a weed species. The objective of the study was to select the monoterpenes with greater phytotoxic activity for further studies on herbicidal properties.

II. MATERIALS AND METHODS

2.1. Chemicals and biological material

Technical grade citronellol, β-pinene and α-terpinene were purchased from Sigma Co., St. Louis, USA. Uniform, healthy seeds of *Cassia occidentalis* were collected locally from plants growing wildly in the campus of Panjab University, Chandigarh, India. Before use *C. occidentalis* seeds were scarified with sulphuric acid and imbibed overnight in water.

2.2. Growth bioassay

Phytotoxicity of all the three monoterpenes was studied on the germination, early growth and chlorophyll content of *C. occidentalis* under laboratory conditions. Monoterpene's solutions (0.50, 1.0 and 2.50 mM) were prepared using Tween-20 (0.01 %). Distilled water with the same amount of Tween-20 served as a parallel control. Pre-imbibed 15 *C. occidentalis* seeds were placed in Petri dishes (15 cm in diameter) lined with a thin layer of cotton wad and Whatman no.1 filter paper. Each Petri dish was moistened with 10 ml of respective solution. The Petri dishes were then sealed with Cello-tape to avoid loss of the monoterpenes due to volatilization. For each treatment concentration, including controls, five independent Petri dishes were maintained as replicates. All the Petri dishes were kept in a growth chamber set at 25 ± 2 °C and 16/8 h light/dark photoperiod of 240 μ mol photons m^{-2} s^{-1} photon flux density provided with fluorescent tubes and lamps. After 7 days, number of seeds germinated, plant growth (in terms of root length, shoot length and seedling dry weight) was measured. Leaves of test plant were taken for further determination of chlorophyll content.

2.3. Estimation of chlorophyll Content

Total chlorophyll content from leaves of test plant (both control and treated) was extracted in dimethyl sulphoxide (DMSO) following the method of [8]. The extinction value was measured at dual wavelength of 645 and 663 nm on Shimadzu UV-1800 double beam spectrophotometer using DMSO as blank. Total chlorophyll content was calculated from extinction values following Arnon's equation [9] and was expressed on dry weight basis.

2.4. Statistical analysis

The experiment was conducted in a completely randomized design with five independent (Petri dish) replicates for each treatment, including control. Statistical analysis of data was done using one-way ANOVA followed by the comparison of mean values using post hoc Tukey's test at $P \leq 0.05$ and finally presented as mean ± SE (Standard Error) using software program SPSS (Version 16.0).

III. RESULTS AND DISCUSSION

Monoterpenes are chemical constituents of plant essential oils. In the present study, inhibitory effects of three commercial monoterpenes including oxygenated monoterpenes and monoterpene hydrocarbons were tested on seed germination, seedling growth and chlorophyll content of *C. occidentalis*. The present results showed that at 2.5 mM citronellol and β-pinene caused a significant reduction in the emergence of coffee weed whereas, α-terpinene show insignificant effect. Monoterpenes show inhibition on germination with this order of potency: citronellol > β-pinene > α-terpinene (Table 1).

Table 1: Effect of different monoterpenes on percent germination of *C. occidentalis* seeds measured after 7 days. Means with the different letters represent significant difference from control at $p \leq 0.05$; ± represent standard error.

Treatment	% Germination at 2.5 mM
Control	100.0 ± 5.4 a
Citronellol	5.8 ± 3.1 b
α-terpinene	97.4 ± 4.6 a
β-pinene	65.3 ± 4.9 b

Not only was the germination but seedling growth, in terms of seedling length and biomass also adversely affected with different degree of inhibition when compared with control (Fig 1, 2 and 3). Citronellol had most significant inhibitory effect on shoot length with approximately 94% reduction in response to 2.5 mM concentration followed by β-pinene and α-terpinene with 24% and 23% respectively over the control (Fig 1).

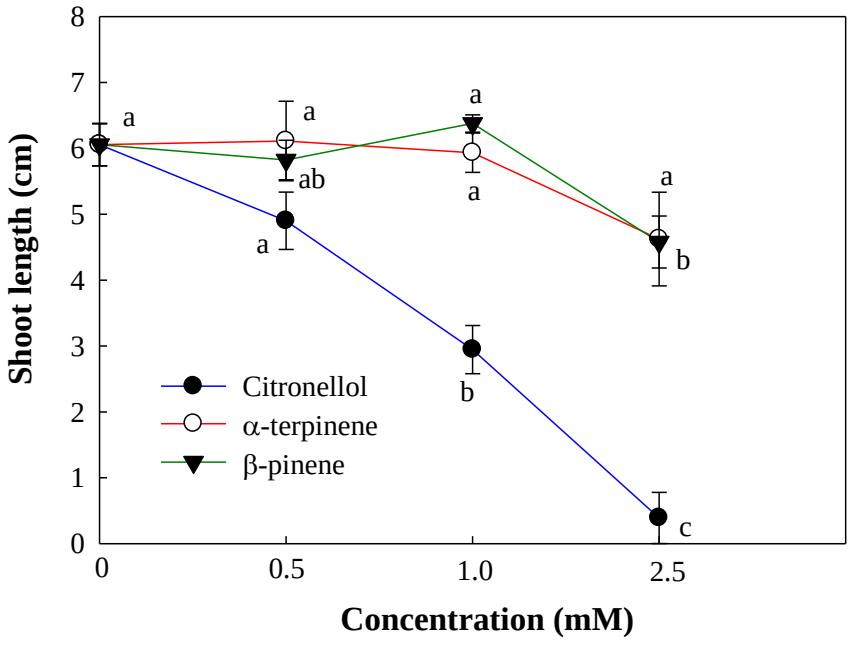

Figure 1. Effect of monoterpenes on shoot length of *C. occidentalis* measured after 7 days. Vertical bars along each data point represent the standard error of the mean and different alphabets along each value represent significant differences from their respective control at $p \leq 0.05$.

International Journal of Advanced Technology in Engineering and Science
Vol. No.5, Issue No. 01, January 2017
www.ijates.com
ijates
ISSN 2348 - 7550

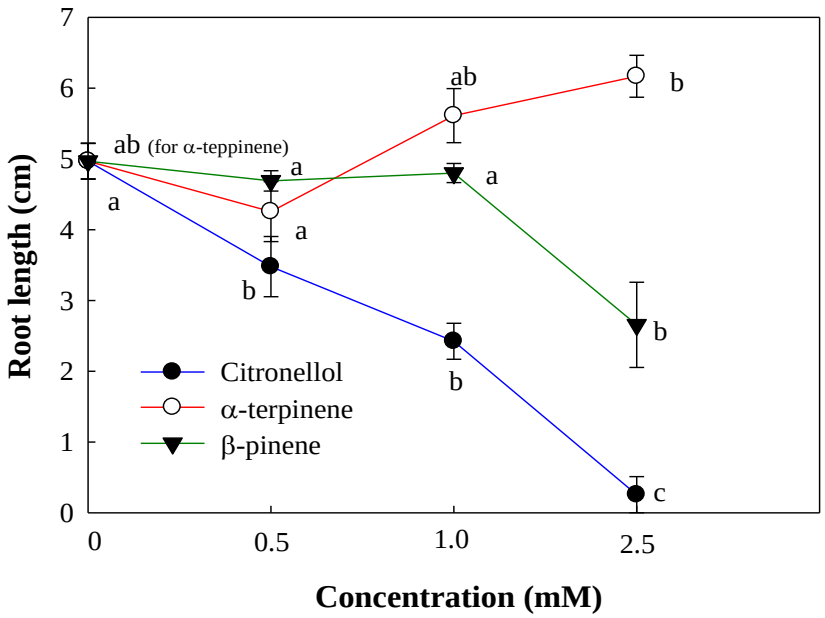

Figure 2. Effect of monoterpenes on root length of *C. occidentalis* measured after 7 days. Vertical bars along each data point represent the standard error of the mean and different alphabets along each value represent significant differences from their respective control at $p \leq 0.05$.

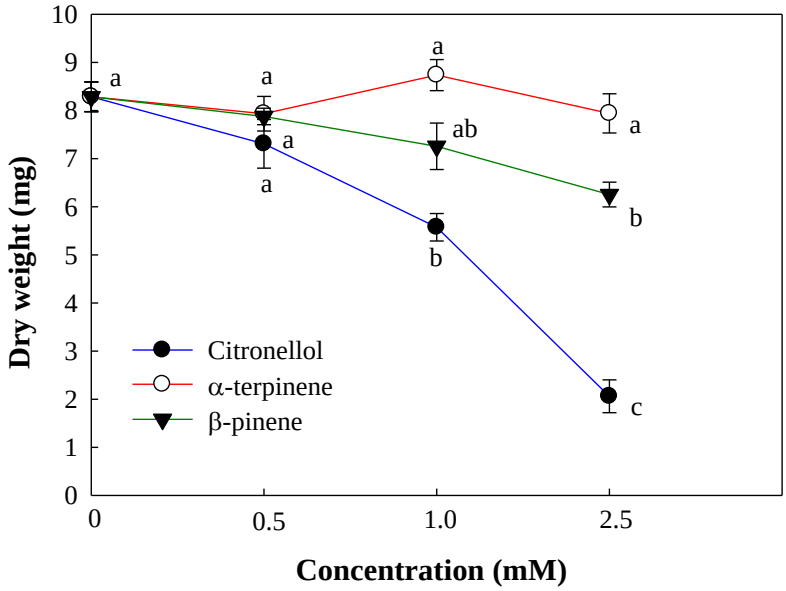

Figure 3. Effect of monoterpenes on dry weight of *C. occidentalis* seedling measured after 7 days. Vertical bars along each data point represent the standard error of the mean and different alphabets along each value represent significant differences from their respective control at $p \leq 0.05$.

Further, inhibition was more prominent for root growth then shoot growth for citronellol and β-pinene with 95% and 46% reduction at 2.5 mM except α-terpinene having promotery effect (Fig 2). Seedling dry weight was significantly reduced by about 75 % and 25 % for citronellol and β-pinene respectively over the control. Hear also α-terpinene showed insignificant reduction (Fig 3). Previous research showed that monoterpenes and essential oils isolated from various plant species possess potent herbicidal effects on weed germination and seedling growth of various plant species [10, 11]. In these researches, monoterpene hydrocarbons showed to

have lower inhibitory effects than oxygenated monoterpenes [11, 12] which is in agreement with present study. The mechanism by which these volatile inhibit seed germination remains unknown. However, loss/disruption of mitotic activity might be responsible for the reduction/inhibition of germination and seedling growth of tested plant [6]. [13] Demonstrated that essential oils/monoterpenes caused accumulation of lipid globules in the cytoplasm and reduced size of cell organelles like chloroplast possibly due to inhibition in DNA synthesis or membrane disruption resulting in anatomical and physiological changes.

Table 2: Effect of different monoterpenes on chlorophyll content (µg/mg DW) of *C. occidentalis* leaves measured after 7 days. Means with the different letters represent significant difference from control at $p \leq 0.05$; ± represent standard error. Values within parenthesis indicate chlorophyll content in % over control

Conc. (mM)	Citronellol	α-terpinene	β-pinene
0.0	11.1 ± 0.9a	11.1 ± 0.9a	11.1 ± 0.9a
	(100 %)	(100 %)	(100 %)
0.5	10.2 ± 0.9a	11.7 ± 0.4a	10.3 ± 0.2ab
	(91.5 %)	(105.1 %)	(92.8 %)
1.0	8.2 ± 0.9ab	10.2 ± 0.4a	9.6 ± 0.2ab
	(74.1 %)	(91.7 %)	(86.1 %)
2.5	5.7 ±0.02b	9.7 ± 0.2a	8.6 ± 0.3b
	(51.4 %)	(86.9 %)	(77.5 %)

Treatment with the monoterpenes also caused a reduction in chlorophyll content of *C. occidentalis* (Table 2). At 2.5 mM of citronellol, the chlorophyll content was reduced by over 49 %, whereas at similar concentrations of β-pinene and α-terpinene, it was reduced by nearly 23 % and 13 %. However, there is no evidence of direct inhibition of chlorophyll synthesis by the monoterpenes in the present study. Nevertheless, the loss of chlorophyll affects photosynthesis. These observations are in conformity with earlier reports that volatiles reduce photosynthetic pigments and thus affect the photosynthetic activity in the plants [14, 11]. However, whether the observed reduction in chlorophyll content was due to decreased synthesis or enhanced degradation of chlorophyll is not known [15]. The relative overall potencies of the monoterpenes tested in our study is citronellol > β-pinene > α-terpinene.

IV. CONCLUSION

The present study therefore concludes that citronellol, on the basis of its overall phytotoxic impact on *C. occidentalis*, is good candidate among all the three monoterpenes for the synthesis of bioherbicide and therefore holds good potential to be used in the future for sustainable weed management programmes.

V. ACKNOWLEDGMENTS

Saroj Kumari Fagodia is thankful to the University Grants Commission, India, for the financial support in the form of fellowship.

REFERENCES

[1] M.W. Aktar, D. Sengupta and A. Chowdhury, Impact of Pesticides Use in Agriculture:Their Benefits and Hazards, Interdisciplinary toxicology, 2, 2009, 1-12.

[2] C.H. Muller, Inhibitory terpenes volatilized from Salvia shrubs, Bulletin of the Torrey Botanical Club, 92, 1964, 38-45.

[3] D. Vokou, Essential oils as allelochemicals: research advances in Greece. Allelopathy Update, Basic and Applied Aspects, Science Publishers, New York, 2, 1999, 47-63.

[4] M.B. Isman, Plant essential oils for pest and disease management, Crop Protection, 19, 2000, 603–608.

[5] L.R. Beuchat, Control of food borne pathogens and spoilage microorganisms by naturally occurring antimicrobials. Microbial Food Contamination, CRC Press, Boca Raton, FL, 2001, 149-169.

[6] J.G. Romagni, S.N. Allen and F.E. Dayan. Allelopathic effects of volatile cineoles on two weedy plant species. Journal of Chemical Ecology, 26, 2000, 303-313.

[7] F. Dayan, J. Romagni, M. Tellez, A. Rimando and S. Duke, Managing weeds with natural products, Pesticide outlook, 5, 1999, 185-188.

[8] T.D. Hiscox and G.F. Israelstam, A method for extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany, 57, 1979, 1332-1334.

[9] D.I. Arnon, Copper enzymes in isolated chloroplasts: Polyphenol oxidase in Beta vulgaris, Plant Physiology, 24, 1949, 1-15.

[10] T. Tworkoski, Herbicide effects of essential oils. Weed Science, 50, 2002, 425-431.

[11] H.P. Singh, D.R. Batish, S. Kaur, H. Ramezani and R.K. Kohli. Comparative phytotoxicity of four monoterpenes against Cassia occidentalis. Annals of applied biology, 141, 2002, 111-116.

[12] D. Vokou, P. Douvli, G.J. Blionis and J.M. Halley, Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. Journal of Chemical Ecology, 29, 2003, 2281-2301.

[13] R. Koitabashi, T. Suzuki, T. Kawazu, A. Sakai, H. Kuroiwa and T. Kuroiwa, 1,8-Cineole inhibits root growth and DNA synthesis in the root apical meristem of Brassica campestris L., Journal of Plant Research, 110, 1997, 1-6.

[14] D.R. Batish, N. Setia, H.P. Singh and R.K. Kohli, Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide, Crop Protection, 23, 2004, 1209-1214.

[15] C.M. Yang, C.N Lee and C.H Chou, Effects of three allelopathic phenolics on chlorophyll accumulation of rice (Oryza sativa) seedlings: I. Inhibition of supply-orientation, Botanical Bulletin of Academia Sinica, 43, 2002, 299–304.