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Terpenoid from essential oil of *Cyperus scariosus* and its biological activity on chilli

Komalpreet Kaur*, Divya Utreja, Pooja Sharma, Seema Bedi and I.S. Grewal

Department of Chemistry, Punjab Agricultural University, Ludhiana, Punjab 141 004

Email: komal-cm@pau.edu

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ABSTRACT

Allelopathic effect of the essential oil of wild sedge- *Cyperus scariosus*, it's polar and non-polar fractions, isolated compound-cyprene and its derivatives: cyprene epoxide and cyprene alcohol were screened for germination studies on chilli (*Capsicum annuum* L.). Chilli is a vegetable crop in Punjab which is having trouble shooting problem during its germination stage, which leads to the interruption in growth curve of the seedling. Treatment of seeds with oil, its fractions and its derivatives lead to stimulatory effect (approx. 75-100%) in terms of primary root and shoot length with collateral increase in dry and fresh weight of seedlings over control at 20 days. Oil and its polar fraction found to be the most effective in enhancing the root length and shoot length, and acts as potent plant growth regulator at the concentration of 0.5 µg/mL.

INTRODUCTION

Cyperus scariosus (Syn. *Cyperus pertenius* Roxb.) known as Cypirol is an invasive weed belongs to family *Cyperaceae* with angular soft stem (40-90 cm), underground rhizomatous tubers, pestiferous perennial, medicinal plant of height 45-75 cm approximately (Srivastava *et al.* 2014). It is commonly known as *Nagarmotha*, and is widely distributed in the forest and swamp areas of tropical and temperate regions of the world (Kasana *et al.* 2013). It is a noxious weed adversely effecting more than 30 crops in 92 countries (Srivastava *et al.* 2014). The weed grows rapidly and fills the soil with its tangle of roots and rhizomes which interfere in the growth of various crops. The presence of chemical constituents such as steroids, alkaloids, terpenoids, saponins, gums, lactones, coumarin, essential oils and esters etc. in *Nagarmotha*, make this weed of immense interest for potential application in various fields (Utreja *et al.* 2015). The essential oil and its various components of weed are known to possess antibacterial, antimicrobial, antifungal, analgesic and antidiabetic, hypotensive, spasmolytic and plant growth regulator activities.

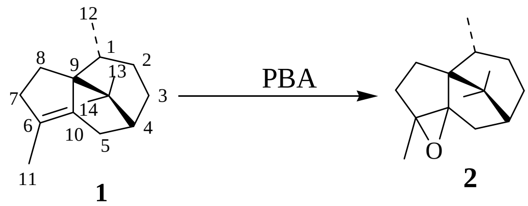
Chilli is an annual herbaceous vegetable crop which belongs to nightshade family Solanaceae. The yield of chilli is very low in third world countries due to lack of good stand establishment and reduced early growth due to adverse environment conditions. Chilli has non-starchy endosperm which offers mechanical barrier to growing embryo resulting in poor germination (Andreoli and Khan 1999, Belakbir *et al.* 1998, Lim *et al.* 2009, Margarita *et al.* 2015). High germination and uniform stand establishment in chilli is essential for profitable yields (Khan *et al.* 2012). It has been found that seed performance of many vegetables can be improved by doing various pre-sowing treatments (Ashraf and Foolad 2005), which may enhance uniform germination, normal and vigorous seedlings resulting in fast and higher rate of germination and emergence (Farooq *et al.* 2007).

Literature citations have shown the positive effects of essential oil of Cypirol and its components as potent plant growth regulator (PGRs) in mung beans (Kalsi *et al.* 1980), grapevine cuttings (Kaur *et al.* 2002), wheat (Sharma *et al.* 2016) and sprouting in sugarcane cuttings (Talwar *et al.* 1999). Therefore, the study was established to evaluate the germination studies of essential oil of *C. scariosus*, it's polar and non-polar fractions, isolated compound-cyprene and its derivatives: cyprene epoxide and cyprene alcohol in chilli.

MATERIALS AND METHODS

C. scariosus oil was subjected to column chromatography to isolate it into non-polar (hexane) and polar fractions (dichloromethane). During column chromatography of oil using hexane as solvent, the non-polar fraction was collected and was distilled with the help of distillation set. Thin layer chromatography (TLC) of compound obtained after distillation from the non-polar fraction was carried out. Silver-nitrate impregnated chromatoplates gave one major spot having R_f = 0.95 and the compound was identified as cyprene **1** on the basis of spectral analysis (Trivedi *et al.* 1964). Epoxidation of cyprene **1** into cyprene epoxide **2** was achieved by using perbenzoic acid (PBA) (Scheme i) and was confirmed with spectral analysis (Sharma *et al.* 2016).

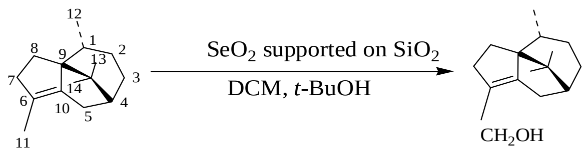
Scheme i: Synthesis of cyprene epoxide **2** from cyprene **1**



1 **2**

Selective oxidation of allylic methyl of cyprene **1** was carried out using selenium dioxide with *t*-butyl hydroperoxide (TBHP) and resulted in the formation of oxidized product cyprene alcohol **3**. In the oxidation procedure selenium dioxide (SeO_2) (2.5 g) was dissolved in minimum quantity of distilled water and volume was raised to 75 mL by addition of methanol to get clear solution. To this clear solution, 50 g of silica gel (60-120 mesh size) was added and was evaporated under reduced pressure to furnish free flowing powder. To selenium dioxide supported on silica gel (0.5 g), dichloromethane (5 mL) and TBHP (3 mL) were added and reaction mixture was stirred for 15 min at room temperature (Scheme ii). To this reaction mixture, dropwise addition of cyprene (1 g) was made and the reaction mixture was allowed to stir at room temperature until the completion of reaction (48 h, TLC). After completion, reaction mixture was filtered and residue was washed with dichloromethane. The filtrate and washings were combined, washed with water, brine, dried over sodium sulfate and evaporation under reduced pressure with rotary evaporator pump, afforded cyprene alcohol **3**. During the reaction procedure the major product was cyprene alcohol (Chhabra and Hayano 1981).

Scheme ii: Synthesis of cyprene alcohol **3** from cyprene **1**



1 **3**

Cyprene alcohol (spectral studies)

IR (KBr): 1382, 1372, 3078, 2953, 3360 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): 1.40 (s, C13-Me), 2.32 (s, C14-Me), 1.23 (d, *J* = 9.2Hz), 4.97 (s, C11- CH_2 attached to –OH group), 8.22 (-OH group)

^{13}C NMR (100 MHz DMSO-d_6): 21.45, 21.95, 25.50, 25.79, 26.40, 26.50, 26.62, 26.82, 29.72, 31.01, 80.96, 135.91, 137.89 ppm.

Products yield: 60%

(s = Singlet, d = doublet, ppm = parts per million, Hz = Hertz and *J* is coupling constant)

The stock solutions (2000 µg/mL) of oil, its polar and non-polar fraction, cyprene **1**, cyprene epoxide **2**, cyprene alcohol **3** were prepared by dissolving each compound (20 mg) in 1 mL of Tween 20 (Polyoxyethylene sorbitan) and volume was made 10 mL with distilled water. The stock solution of 2000 µg/mL of each compound thus prepared on active ingredient basis and was kept in refrigerator till use. The required dilutions of 100, 75, 50, 25, 20, 15, 10, 5, 2.5, 1, 0.5 µg/mL were subsequently made from the stock solution by adding distilled water as and when required.

Chilli seeds were obtained from the Department of Vegetable Sciences, Punjab Agricultural University, Ludhiana. Ten homogenous seeds were plated per Petri-dish (9 cm) lined with Whatman filter paper (pure cellulose paper) and moistened with solutions of the oil, polar fraction, non-polar fraction, cyprene and cyprene alcohol at different concentrations (0.5–100 µg/mL) along with distilled water as control. 6 mL solution of each compound was poured in each Petri plate regularly to soak seeds. The seeds were not let dry and the experiment was replicated thrice for each treatment. The Petri-dishes containing seeds were incubated at 28±2 ^{0}C and were observed daily for twenty days for germination. Germinated seeds were observed and counted daily. The appearance of 2 mm or more of radicle was considered as germination and germination percentage was calculated as follow:

Germination percentage (GP) = (Total germinated seed/ total number of seed) x 100

The morphological characters such as root length and shoot length were measured at the end of twenty days after plating with a graduated meter. At the end of twenty days, fresh weight of seedlings was recorded and then the seedlings were oven dried at 60 ^{0}C for 3 days for their dry matter content. Various morphological parameters of chilli recorded in distilled water as control were root length (1.5 cm), shoot length (2.3 cm), fresh weight (22 mg) and dry weight (9 mg). Shoot length was measured from the base till tip of the uppermost leaf. Root length was measured from the base till tip of the longest root.

The results of the enhancement of the plant growth were calculated in the terms of seedling vigour index.

Seedling vigour index = % germination × total seedling dry weight

The statistical analysis for the plant growth regulator activity of the oil, its polar and non-polar fractions, isolated compounds cyprene **1** and its derivatives cyprene epoxide **2**, cyprene alcohol **3** was carried out on seedlings of chilli. The compounds (A), concentrations (B) and their interactions were studied statistically by Factorial CRD and Tukey's test to study the extent of dependency of concentrations and compounds and their effect on enhancement of root length, shoot length, fresh weight, dry weight and seedling vigour index of chilli seedlings.

The seedling growth parameters *viz.* root length and shoot length, fresh and dry weight were recorded at the end of 20 days after placing the seeds for germination.

RESULTS AND DISCUSSION

The seeds treated with polar fraction concentrations (0.5 µg/mL) showed 100% germination, which also initiated early germination. In other test fractions, higher germination percentage (75%) was observed when treated with more than 2.5 µg/mL concentration. The number of roots were found to be four or five in the *C. scariosus* oil at each concentration. But in case of other compounds only two or three roots were observed. In control, only one root was observed. Therefore, *C. scariosus* oil was effective in growth of secondary roots.

Both shoot and root length, significantly increased with the increase in concentration of each compound. Polar fraction was found to be most active in enhancing shoot length (**Table 1**) and it was found that there is significant interaction between A and B. All the compounds showed significantly different activity with respect to each other but the activity of Cyprene **1** was found to be at par with non-polar fraction (**Table 6 and 7**).

C. scariosus oil was found to be the most active in enhancing root length (**Table 2**). But in root length analysis, there was significant interaction between A and B, all compounds showed significantly variant activity from each other. Cyprene **1**, cyprene epoxide

Table 1. Effect of oil and its components on shoot length (cm)

Conc. (µg/mL)	Oil	Non-polar	Polar	Cyprene	Cyprene epoxide	Cyprene alcohol
0.5	1.46	0.96	1.70	1.06	1.43	1.60
1.0	1.93	1.13	2.00	1.30	1.53	1.80
2.0	2.03	1.20	2.36	1.30	1.53	2.20
2.5	2.10	1.43	2.60	1.53	1.86	2.40
5.0	2.26	1.50	2.90	1.60	2.00	2.70
10.0	2.20	1.50	3.13	1.90	2.20	2.70
20.0	2.50	1.60	3.80	2.00	2.30	3.00
25.0	2.60	1.80	4.00	2.10	2.40	3.20
50.0	3.10	2.10	4.90	2.40	2.60	3.90
75.0	3.20	2.30	5.90	2.50	2.70	4.00
100.0	3.30	2.40	6.00	2.90	3.10	4.20
Particulars			LSD (p=0.05)			
A			0.027			
B			0.019			
AB			0.066			
CV			1.68			

Table 2. Effect of oil and its components on root length (cm)

Conc. (µg/mL)	Oil	Non-polar	Polar	Cyprene	Cyprene epoxide	Cyprene alcohol
0.5	3.76	1.50	3.53	1.73	2.70	3.06
1.0	3.90	1.70	3.56	1.90	2.86	3.20
2.0	4.10	1.83	3.80	2.00	2.90	3.40
2.5	4.48	1.90	3.93	2.10	3.00	3.60
5.0	4.70	2.00	3.86	2.30	3.06	3.70
10.0	5.00	2.16	4.16	2.70	3.20	3.70
20.0	5.23	2.40	4.23	2.86	3.30	3.80
25.0	5.26	2.50	4.46	3.00	3.43	4.00
50.0	5.30	2.66	4.70	3.10	3.73	4.10
75.0	5.60	3.00	4.93	3.30	3.90	4.30
100.0	5.69	3.16	5.10	3.56	4.00	4.70
Particulars			LSD (p=0.05)			
A			0.027			
B			0.020			
AB			0.068			
CV			1.21			

2, cyprene alcohol **3**, and non-polar fraction also showed potential in enhancing the shoot and root length of the seedlings.

Likewise, fresh weight of the seedlings showed an increasing trend with the increasing concentration of the compounds as there is significant interaction between A and B. Statistically, all compounds showed significantly variant activity from each other but activity of Cyprene and non-polar fraction did not depict significant difference. Oil showed maximum effect in increasing the fresh weight of the seedlings at higher concentrations followed by cyprene alcohol **3** (**Table 3**).

A similar trend was observed in dry weight of seedling. Dry weight of the seedlings showed an increasing trend with the concentration of the compounds as there is significant interaction between A and B. All the compounds showed significantly variant activity from each other. Polar fraction

Table 3. Effect of oil and its components on fresh weight (mg)

Conc. (µg/mL)	Oil	Non-polar	Polar	Cyprene	Cyprene epoxide	Cyprene alcohol
0.5	35.31	19.27	29.24	21.34	31.21	33.04
1.0	36.30	20.25	29.22	21.31	32.20	34.05
2.0	38.28	20.23	30.20	22.29	34.17	36.07
2.5	39.24	21.20	32.18	24.26	35.06	37.08
5.0	41.22	23.19	33.16	25.24	36.21	39.09
10.0	42.20	24.17	34.14	26.21	37.20	40.11
20.0	44.18	27.16	36.13	26.20	39.08	41.13
25.0	45.17	29.15	39.12	28.19	40.14	43.14
50.0	47.16	31.13	42.11	30.17	44.20	44.17
75.0	49.14	33.12	43.10	31.16	45.25	45.20
100.0	51.13	36.11	45.10	32.15	46.33	47.21
Particulars			LSD (p=0.05)			
A			0.138×10^{-1}			
B			0.102×10^{-1}			
AB			0.338×10^{-1}			
CV			0.03			

Table 4. Effect of oil and its components on dry weight (mg)

Conc. (µg/mL)	Oil	Non-polar	Polar	Cyprene	Cyprene epoxide	Cyprene alcohol
0.5	10.01	4.15	15.54	6.00	11.45	13.31
1.0	10.40	5.50	16.69	7.25	12.64	14.30
2.0	11.11	7.96	17.33	9.16	13.72	16.30
2.5	12.58	8.54	19.75	11.85	15.68	17.24
5.0	13.02	9.38	20.25	12.62	16.65	18.28
10.0	14.25	11.58	21.92	13.23	17.98	20.03
20.0	16.30	13.18	24.83	14.21	19.39	22.58
25.0	18.18	14.96	26.77	15.04	20.42	24.95
50.0	20.00	17.28	29.58	18.98	23.25	28.65
75.0	22.75	20.33	31.34	20.26	25.00	30.00
100.0	24.54	21.69	34.21	21.00	27.01	31.54
Particulars			LSD (p=0.05)			
A			0.784×10^{-2}			
B			0.579×10^{-2}			
AB			0.019×10^{-1}			
CV			0.07			

showed maximum effect in increasing the dry weight of the seedlings at higher concentrations followed by cyprene alcohol **3** (**Table 4**).

The results of seedling vigour index showed that the polar fraction was found to be effective in improving the performance of the seeds in terms of germination followed by cyprene alcohol **3**, cyprene epoxide **2,** oil, cyprene **1** and its non-polar fractions (**Table 5**). But the oil and cyprene epoxide **2** showed no significant difference in their activities. The interaction between A and B was not found to be significant in seed vigour index. In an earlier study (Kaur *et al.* 2002), an investigation was done on the effect of *C. scariosus* oil on rooting, sprouting and accompanying biochemical changes in grapevine cuttings. An enhancement in number of primary roots, length of longest primary root, shoot length and also total dry weight of roots and shoots were observed over control (Kaur *et al.* 2002). Similarly,

Table 5. Effect of oil and its components on seedling vigour index

Conc. (µg/mL)	Oil	Non-polar	Polar	Cyprene	Cyprene epoxide	Cyprene alcohol
0.5	0.950	0.249	1.554	0.450	0.916	1.197
1.0	0.988	0.330	1.669	0.543	1.011	1.287
2.0	1.055	0.477	1.733	0.687	1.097	1.467
2.5	1.195	0.512	1.975	0.888	1.254	1.551
5.0	1.236	0.568	2.025	0.946	1.332	1.645
10.0	1.353	0.694	2.192	0.992	1.438	1.802
20.0	1.548	0.790	2.483	1.065	1.551	2.032
25.0	1.727	0.897	2.677	1.128	1.633	2.245
50.0	1.900	1.036	2.958	1.423	1.860	2.578
75.0	2.161	1.219	3.134	1.519	2.000	2.700
100.0	2.331	1.301	3.421	1.575	2.160	2.838
Particulars			LSD (p=0.05)			
A			0.029×10^{-5}			
B			0.154×10^{-5}			
AB			NS			
CV			0.003			

Table 6. Compounds with corresponding effective concentration order

Compound with effective concentration order				
Polar (Shoot length)	Oil (Root length)	Oil (Fresh Weight)	Polar (Dry Weight)	Polar (Seed Vigour index)
0.5^{h}	0.5^{d}	0.5^{g}	0.5^{f}	0.5^{f}
1.0^{hg}	1.0^{d}	1.0^{fg}	1.0^{f}	1.0^{ef}
2.0^{fg}	2.0^{cd}	2.0^{efg}	2.0^{f}	2.0^{ef}
2.5^{fe}	2.5^{bcd}	2.5^{ef}	2.5^{e}	2.5^{def}
5.0^{de}	5.0^{abcd}	5.0^{de}	5.0^{de}	5.0^{de}
10.0^{d}	10.0^{abc}	10.0^{de}	10.0^{d}	10.0^{cd}
20.0^{c}	20.0^{ab}	20.0^{cd}	20.0^{c}	20.0^{bc}
25.0^{c}	25.0^{ab}	25.0^{cc}	25.0^{c}	25.0^{cd}
50.0^{b}	50.0^{ab}	50.0^{bc}	50.0^{b}	50.0^{bc}
75.0^{a}	75.0^{a}	75.0^{ab}	75.0^{b}	75.0^{ab}
100.0^{a}	100.0^{a}	100.0^{a}	100.0^{f}	100.0^{a}

Tukey's Test (General linear model, Univariate, SPSS Software, 16.0):

*Compounds with similar alphabets are non-significant to each other and compounds with different alphabets are significant to each other.

effect of *C. scariosus* oil and its various fractions was studied on germination and seedling establishment in two cultivars of wheat. A promontory effect was reported on seed germination, number and length of roots, seedling dry weight and seedling vigour index (Sharma *et al.* 2016).

In the present study, the decreasing order of activity at different concentrations of *C. scariosus* oil, polar fraction, non-polar fraction, cyprene, cyprene epoxide and cyprene alcohol was as follows:

Polar fraction > *C. scariosus* oil > Cyprene alcohol > Cyprene epoxide > Cyprene > Non-polar fraction.

Table 7. Compounds with corresponding activity order

Parameter	Compound					
Shoot length	Oil[c]	Non-polar[e]	Polar[a]	Cyprene[e]	Cyprene epoxide[d]	Cyprene alcohol[b]
	2.39	1.56	3.78	1.76	2.27	2.98
Root length	Oil[a]	Non-polar[f]	Polar[b]	Cyprene[e]	Cyprene epoxide[d]	Cyprene alcohol[c]
	5.12	1.13	4.25	2.77	3.28	3.75
Fresh weight	Oil[a]	Non-polar[e]	Polar[d]	Cyprene[e]	Cyprene epoxide[c]	Cyprene alcohol[b]
	42.57	25.90	35.81	26.22	38.27	39.66
Dry weight	Oil[d]	Non-polar[f]	Polar[a]	Cyprene[e]	Cyprene epoxide[c]	Cyprene alcohol[b]
	15.74	12.23	23.47	13.60	18.47	21.56
Seed vigour index	Oil[c]	Non-polar[e]	Polar[a]	Cyprene[d]	Cyprene epoxide[c]	Cyprene alcohol[b]
	1.35	0.63	2.17	0.92	1.37	1.78

Tukey's Test (General linear model, Univariate, SPSS Software, 16.0):
*Compounds with similar alphabets are non-significant to each other and compounds with different alphabets are significant to each other.

The extract from *C. scariosus* plant has multiple pharmacological uses (Utreja *et al*. 2015). The present study and earlier reports also indicate plant growth regulator effects of *C. scariosus* oil.

From the germination studies on *C. annuum* L., it was concluded that the essential oil of *C. scariosus*, its polar and non-polar fraction, isolated compound-cyprene **1** and its derivatives-cyprene epoxide **2** and cyprene alcohol **3** have significant effect on the seedling growth parameters such as root length, shoot length, fresh weight and dry weight along with seed vigour index and act as promising plant growth regulator.

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