Phytotoxic selectivity analysis of *Eucalyptus tereticornis* essential oil against rice, *Oryza sativa* and its weeds, *Echinochloa crus-galli* and *Cyperus rotundus*

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ABSTRACT

The present study was undertaken to explore the phytotoxic potential of essential oil (EO) from *Eucalyptus tereticornis* against two major weeds of rice viz *Echinochloa crus-galli* and *Cyperus rotundus*. The effect of essential oil was studied on growth (percentage germination, root length and shoot length development) physiological parameters (chlorophyll content and per cent respiration) under controlled conditions in the laboratory. Studies revealed that *E tereticornis* essential oil (in different concentrations ranging from 25 to 250 μ g/ml) affected the growth and physiology of all the test plants. However the effect was more predominant on weeds compared to the rice seedlings. When the seeds of test plants were exposed to 100 and 250 μ g/ml oil concentration seed germination and seedling development of test weeds were highly affected as compared to rice. *C rotundus* was found to be the most sensitive weed among test plants. In chlorophyll content the reduction at 250 μ g/ml of essential oil was 60, 80 and 99 per cent in *O sativa*, *E crus-galli* and *C rotundus* respectively. Similarly reduction in respiratory activity on exposure to 250 μ g/ml of essential oil was 20, 40 and 99 per cent in *O sativa*, *E crus-galli* and *C rotundus* respectively. The present study concludes that essential oil of *E tereticornis* shows the toxicity selectively towards the *E crus-galli* and *C rotundus* compared to *O sativa*.

Keywords: Essential oil; weeds; rice; toxicity; bio-herbicide

INTRODUCTION

The productivity of rice is severely affected by weeds in the agro-ecosystem. The major weeds of the crop are Echinochloa crus-galli L, Cyperus rotundus L, Echinochloa colonum L, Eptochloa chinensis L, Ischaemum rugosum, Aeschynomene aspera L,

Cynodon dactylon L. Holm et al (1977), Fischer et al (2000) and Sudianto et al 2013 have reported *E crus-galli* and *C rotundus* commonly known as Cockspur or Cockspur grass or Barnyard grass and Coco grass respectively as the worst weeds of rice. The continuous and arbitrary use of synthetic and chemical herbicides to control these has led to multiple toxic

effects on the ecology and environment (Tsui and Chu 2003, Relyea 2005, Contardo-Jara et al 2009).

During the last 40 years a number of researchers have highlighted the potential importance of natural plant products as herbicides (Komai and Tang 1989, Duke et al 1997, Smith et al 1997, Mitra et al 2001, Leo et al 2002, Abad et al 2007). Among the natural plant products volatile essential oils are known to possess relatively high phytotoxicity towards a number of plants (Kohli and Singh 1991, Dudai et al 1999, 2004, Tworkoski 2002, Batish et al 2004, Singh et al 2005, Cavalieri and Caporali 2008, Verdeguer et al 2011). Among various aromatic plants *Eucalyptus* sp is well known for its essential oil (EO) because of higher yield and easy availability. Among various species of *Eucalyptus* the EO of *E tereticornis* is reported to have antioxidant (Singh et al 2009), pesticidal, nematicidal and fungicidal properties (Arango et al 2010).

The present work was planned to check the phytotoxicity of EO from the leaves of *E tereticornis* against rice (*O sativa*) and its two weed species *E crusgalli* and *C rotundus* with the objective of exploring its possible use as a bio-herbicide.

MATERIAL and METHODS

Collection of Plant materials: The leaves of *E tereticornis* were collected from the

field near Central University of Punjab, Bathinda (30.1700° N, 76.4500° E) during Nov-Dec 2012. Seeds of *E crus-galli* and *C rotundus* were collected from Punjab Agricultural University, Ludhiana, Punjab.

Extraction of volatile oil: Essential volatile oil from fresh and healthy leaves of *E tereticornis* was extracted by steam distillation using Clevenger's apparatus. For this the leaves were chopped into pieces and mixed with distilled water and placed in the steel container of Clevenger's apparatus fitted with condenser. Mixture was boiled for 2 h and oil was collected.

Composition of essential oil: The composition and the identification of the EO were studied using gas chromatograph coupled with mass spectrophotometer (Shimadzu OP 2010 Mass Spectrophotometer). The compounds were identified by comparing the mass spectra of components with their reference spectra and matching the Kovats/retention indices (RI) with reference to homologous series of n-alkanes (C_7 - C_{30} Supelco, Bellefonte, PA, USA). Some compounds were searched as per Adams (2007). Kovats retention index of each component was determined by co-injection of oil with homologous series of *n*-alkanes (C_8 - C_{32}) under exactly the same conditions.

Growth studies: To study the effect of EO on seeds germination, protocol of Azirak and Karaman (2008) was adopted with

slight modifications. Seeds of E crus-galli and C rotundus were dipped in distilled water for overnight and were sown in 15 cm diameter Petri dishes lined with two layers of moistened Whatman no 1 filter paper. An aliquot of 0, 0.5, 1.5, 2.5, and 5.0 µl of EOs was applied on the inner side of the cover of Petri dish. Immediately after the treatment each dish with its cover was sealed with a piece of para film to reduce evaporation. A similar set without EOs served as control. The experiment was replicated thrice and kept for one week at 25±2°C for 12 hours in dark and for 12 hours in light condition. The study was carried in terms of per cent germination, measurement of root and shoot length and dry weight of 7 days old seedlings.

Estimation of chlorophyll content:

Chlorophyll from 25 mg of treated and control leaves was extracted in 4 ml of dimethyl sulphoxide (DMSO) as per the method of Hiscox and Israelstam (1979). It was quantified spectrophotometrically using the equation of Arnon (1949) and expressed on dry weight basis as suggested by Rani and Kohli (1991).

Biochemical parameter analysis: Plant material was freed of pigment, fats and lipids by dissolving in acetone (for 72 hours) followed by acetone/petroleum ether (1:1, for 24 hours) and finally in petroleum ether (for 24 hours). It was powdered and used for estimation of carbohydrate and protein content. Estimation of carbohydrate content

was done as per method of Loewus (1952) and of protein content by the method of Lowry et al (1951).

All the experiments were performed in a completely randomized block design and the results were reproduced twice. The data collected from dose response study were subjected to one way ANOVA with Tukey's test.

RESULTS

Composition of essential oil: The major constituents of essential oil were studied by using gas chromatography coupled with mass spectrophotometer. Result obtained from the GC-MS analysis showed that the oil was a mixture of monoterpines, sesquiterpenes, alcohols, ketons etc. In all 33 components were identified constituting around 99.28 per cent of the total (Table 1).

Majority of the constituents were monoterpene constituting approximately 72 per cent of the oil. The 3 unidentified constituents α -Pinene (34.47%), 1,8-Cineole (25.14%) and β -Pinene (12.94%) were the major components constituting more than 50 per cent of the oil. Apart from these the other constituents in high concentration were α -Eudesmol (2.43%) and β -Eudesmol (3.82%).

Germination percentage: It was observed that the essential oil from E

Table 1. Composition of essential oil from leaves of *E tereticornis*

Retention time	Compound	Constituent (%)	Chemical classification	Kovats index
2.597	α-Pinene	34.47	МН	1056
2.882	α-Thujene	0.13	МН	1076
2.967	Camphene	0.26	МН	1083
3.417	β -Pinene	12.94	МН	1114
3.625	Sabinene	0.05	МН	1127
4.191	β -Myrcene	0.18	МН	1163
4.245	α -Phyllandrene	1.29	МН	1167
4.865	Limonene	3.56	МН	1204
5.079	1,8-Cineole	25.14	OM	1213
5.884	α -Terpinene	0.43	МН	1246
6.501	Cymene	1.21	МН	1272
6.782	Terpinolene	0.10	МН	1284
12.676	β -Citronellal	1.99	OM	1480
15.168	Isopulegol-I	0.49	OM	1560
15.438	Isopulegol-II	0.45	OM	1569
15.883	endo-Fenchol	0.44	OM	1584
15.956	trans- β -Caryophyllene	0.36	SH	1586
16.236	Aromadendrene	0.26	SH	1595
16.406	Terpinen-4-ol	0.48	OM	1601
17.988	trans-Pinocarveol	1.75	OM	1654
18.316	Citronellyl acetate	0.27	OM	1665
19.169	α -Terpineyl acetate	0.51	OM	1693
19.325	β -Terpineol	3.27	OM	1698
21.475	1,8 - Citronellol (R)	0.71	OM	1772
22.067	Myrtenol	0.53	OM	1793
29.458	Globulol	0.24	OS	2069
31.835	γ-Eudesmol	0.85	OS	2166
32.226	Aristolene	0.11	OS	2182
32.299	Hinesol	0.16	OS	2185
33.061	α -Eudesmol	2.43	OS	2217
33.263	β -Eudesmol	3.82	OS	2225

MH: Monoterpene hydrocarbons OM: Oxygenated monoterpenes SH: Sesquiterpene hydrocarbons OS: Oxygenated sesquiterpenes

tereticornis affected the seed germination of test plants *O sativa*, *E crus-galli* and *C rotundus*. At lowest concentration (25 μg/ml) no effect was observed on *O sativa* seeds while in *E crus-galli* and *C rotundus* significant decrease was observed. At highest concentration of 250 μg/ml the highest reduction was observed in *C rotundus* (approx 91%) followed by *E crus-galli* (almost 70%). *O sativa* was the least affected even at highest concentration of 250 μg/ml the per cent reduction in germination being 35 per cent (Fig 1).

The results clearly indicate that the affect of oil treatment was more on the weed seeds compared to the crop seeds. Batish et al (2004, 2006, 2007, 2008) reported similar observation in which they found that the essential oils are potent inhibitor of germination of weed species as compared to the essential crops.

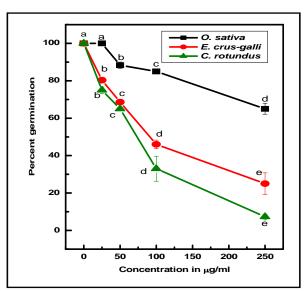
Effect on shoot length: The essential oil of *E tereticornis* affected the development of shoot length of test plants. At lowest concentration of 25 μ g/ml no effect was observed on *O Sativa* seeds while in *E crus-galli* and *C rotundus* significant decrease was observed. At higher concentration of 250 μ g/ml the highest reduction was observed in *C rotundus* (approx 99%) followed by *E crus-galli* (almost 80%) (Fig 2).

O sativa was the least affected even at highest concentration (250 µg/ml) and the

reduction in shoot length was 65 per cent. This indicates that the crop seeds were more tolerant to the toxicity induced by the essential oil.

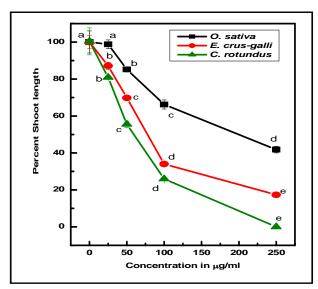
On the basis of selectivity ratio analysis it was concluded that the essential oil was more toxic to *C rotundus as* compared to *E crus-galli*. At 100 µg/ml essential oil was highly toxic to the weeds as compared to rice. Therefore this concentration can be used for developing the herbicidal formulation of essential oil.

Effect on root length (Fig 3): In case of rice at lowest concentration of EO (25 µg/ ml) the reduction was negligible and at highest concentration (250 µg/ml) it was up to 40 per cent. While in the case of Ecrusgalli and C rotundus 20 and 40 per cent reduction was observed at lower concentration of 25 µg/ml. In C rotundus development of root was highly affected in response to treatment of essential oil and at higher concentration of 250 µg/ml no roots were developed. Similar results were observed by Singh et al (2005), Kordali et al (2008) and Chowhan et al (2011). Muller (1965) reported that volatile oils from Salvia leucophyalla inhibit the mitosis in root and shoot tips of Cucumis sativus and inhibit the growth of roots and shoots. In another study Nishida et al (2005) reported that the monoterpenoids produced by S leucophylla interfere with the growth of other plants in its vicinity by blocking of cell division in the root and shoot apical



Different alphabets along each line represent significant differences over control at $P \le 0.05$ applying Tukey's test, a= Not significant, b <0.05, c <0.01, d <0.001, e <0.0001

Fig 1. Effect of essential oil of *E tereticornis* on the germination of *O sativa*, *E crus-galli* and *C rotundus*



Different alphabets along each line represent significant differences over control at $P \le 0.05$ applying Tukey's test, a= Not significant, b <0.05, c <0.01, d <0.001, e <0.0001

Fig 2. Effect of essential oil of E tereticornison the shoot length of O sativa, E crus-galliand C rotundus

meristems. Similar observations have been made by Batish et al (2007) and Singh et al (2009).

Effect on dry weight (Fig 4): At low concentration of $25 \mu g/ml$ the decrease in dry weight of *O sativa* was insignificant while significant decrease was observed in the test weeds. At highest concentration ($250 \mu g/ml$) the loss of dry weight was much higher up to 80-99 per cent in both the weeds while in *O sativa* it decreased only up to 35 per cent. *Eucalyptus* sp essential oil showed same effect on different weeds like *A hybridus* and *P oleraca* (Verdeguer et al 2011).

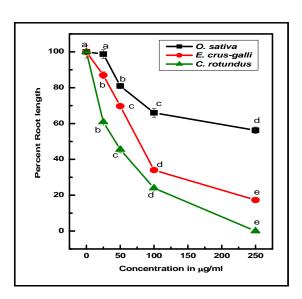
Effect on total chlorophyll content: In general the chlorophyll content decreased with increase in concentration of oil (Fig 5). The effect was even significant at lowest concentration on the test crop and test weeds. At 100 µg/ml concentration there was a significant reduction of approximately 40 per cent in O sativa, 60 per cent in E crus-galli and 80 per cent in C rotundus. At highest concentration of 250 µg/ml the chlorophyll content was reduced up to 60, 80 and 99 per cent in O sativa, E crus-galli and C rotundus respectively. The similar observations for chlorophyll content were reported by different workers (Tworkoski et al 2002, Setia et al 2007, Kaur et al 2010).

Effect on cellular respiration: In *O sativa* very little decrease in respiration was

observed at highest concentration of EO (250 µg/ml) as compared to control while in case of C rotundus the reduction was 40 and 80 per cent with increase in concentration from 50 and 100 µg/ml and in E crus-galli it was up to 20 and 40 per cent respectively (Fig 6). Earleir workers reported that volatile oil from Eucalyptus spp (E citriodora, E globulus) reduced the respiration process in Parthenium hysterophorus, Triticum aestivum, Zea mays, Raphanus sativus, Cassia occidentalis, Amaranthus viridis and Echinochloa crus-galli (Kohli et al 1998, Mucciarelli et al 2001, Batish et al 2004, 2007, Singh et al 2005, Setia et al 2007).

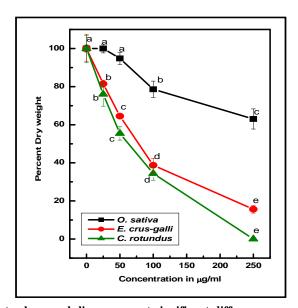
DISCUSSION

The seed germination, seedling length and dry weight of the test plants were significantly affected in response to vapours of essential oil from leaves of Etereticornis plant. In general a decrease was observed with increase in concentration of oil. At highest concentration decrease in germination, seedling length and dry weight of the test plants was observed. However the effect was variable in the two test weeds. This can be attributed to the fact that the seeds with soft outer seed covering like C rotundus were more affected as compared to seeds of O sativa and E crus-galli with hard seed coats. Further the seeds with small size were more affected to the treatments compared to seeds with bigger size. Seeds of C rotundus being small in size were more affected as compared to O



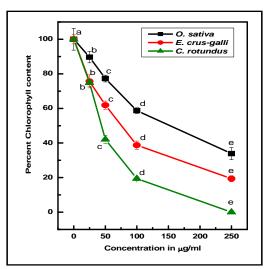
Different alphabets along each line represent significant differences over control at $P \le 0.05$ applying Tukey's test, a= Not significant, b <0.05, c <0.01, d <0.001, e <0.0001

Fig 3. Effect of essential oil of *E tereticornis* on the root length of *O sativa*, *E crus-galli* and *C rotundus*



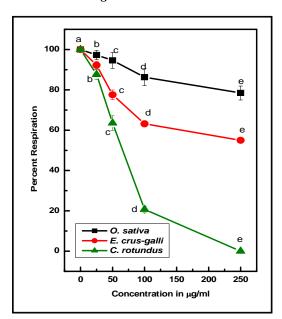
Different alphabets along each line represent significant differences over control at $P \le 0.05$ applying Tukey's test, a= Not significant, b <0.05, c <0.01, d <0.001, e <0.0001

Fig 4. Effect of essential oil of E tereticornis on the percent dry weight of O sativa, E crus-galli and C rotundus



Different alphabets along each line represent significant differences over control at $P \le 0.05$ applying Tukey's test, a= Not significant, b <0.05, c <0.01, d <0.001, e <0.0001

Fig 5. Effect of essential oil of E tereticornison per cent chlorophyll of O sativa, E crus-galli and C rotundus



Different alphabets along each line represent significant differences over control at $P \le 0.05$ applying Tukey's test, a= Not significant, b <0.05, c <0.01, d <0.001, e <0.0001

Fig 6. Effect of essential oil of E tereticornison the per cent respiration of O sativa, E crus-galli and C rotundus

sativa and E crus-galli which have comparatively larger seeds. Hence with increase in seed size and weight the effect of the treatment decreased. Similar studies regarding phytotoxicity of essential oil of a number of plants and their pure components have earlier been reported (Ponce et al 2004, Singh et al 2002, Angelini et al 2003, Vokou 2003, Batish et al 2007, Kordali et al 2008, Singh et al 2009, Zanellato et al 2009, Kaur et al 2010, Chowhan et al 2011). In the present study greater inhibition in germination and growth was observed in test weeds as compared to test crop. This shows the specific phytotoxic potential of E tereticornis essential oil against the test weeds. This differential effect may be attributed to genetic factors, morphology of seeds and also the seed size. The mechanism of action of essential oil or its pure components include mitotic inhibition, inhibition of protein metabolism, changes in hormones and root tip inhibition interfering with photosynthesis activity (Ahmed and Wardle 1994, Ibrahim et al 2001, Singh et al 2002, Nishida et al 2005, Singh et al 2006, Bainard et al 2006, Bisio et al 2010, Mutlu et al 2010).

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