Bioherbicidal potential of essential oil from leaves of *Eucalyptus tereticornis* against *Echinochloa crus - galli* L.

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ABSTRACT

The present study was undertaken to explore the bioherbicidal potential of essential oil(EO)(25 to 250µg/ml) from *Eucalyptus tereticornis* against one of the major weed of rice (*Oryza sativa* L.), *i.e. Echinochloa crus – galli* L. considering percent germination, root length and shoot length development chlorophyll, protein and carbohydrate content and percent cellular respiration). Studies revealed that *E. tereticornis* EO suppressed the growth and affects the physiology of the test plant. For instance, 100 and 250µg/mL oil affects seed germination and seedling development of test weed. The chlorophyll content of the *E. crus- galli* seedlings decreased by 80% at250µg/mL treatment of EO. Similarly, reduction in respiratory activity on exposure to 250µg/mL of EO was 60%. The effect of EO on macromolecules, *i.e*. carbohydrates and proteins also followed the similar trend. The present study concludes that EO of *E. tereticornis* shows toxicity towards *E. crus- galli* and has potential to be used as bioherbicide.

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INTRODUCTION

Rice (*Oryza sativa* L.), a member of the Oryzoid group is one of the most important staple food for humans throughout the world. Among various Asian countries, China and India are the major producers of this crop (IRRI, 2011). In India, it is cultivated in nearly all states but the major producers are Punjab, Haryana, Uttar Pradesh, Bihar, and West Bengal. The main varieties cultivated are Jaya, Pusa Sugandh, Unnatt Pusa, Basmati-1, Pusa R. H. 10. Due to increase in population, the demand for rice is growing every year. As per estimates by (Thiyagarajan and Selvaraju, 2001; Ganesh Kumar *et al.,* 2012) the current demand of rice is approximately 145 million tons and it is estimated that it will reach up to 169 million ton in 2025. So in order to sustain present food self-sufficiency and future food requirements, India has to increase its rice productivity by 3 percent per annum. However, the productivity of rice is severely affected by weeds in the agro-ecosystems. 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. (Johnson *et al*., 2003). Holm *et al*. (1977) has reported *Echinochloa crus - galli* L. Cockspur or Cockspur Grass or Barnyard Grass as the worst weed of rice. Further, Fischer *et al*. (2000) and Sudianto *et al*. (2013) considered *E. crus-galli* is worst weeds of rice because of the following reasons: Of the various methods of weed control, the chemical methods are more preferred worldwide because of their easy applicability and quick action. The continue and indiscriminate use of synthetic and chemical herbicides in agriculture has led to multiple toxic effects on ecology and environment (Tsui and Chu, 2003; Relyea, 2005; Rassaeifar *et al.,* 2013).Therefore, there is an urgent need to find alternative methods or chemicals which should be environmentally safe, readily biodegradable in nature, more target specific, multiple sites for their action to prevent resistance and cost effective.

During the last 40 years, a number of researchers has highlighted the potential importance of natural plant products as herbicides (Duke *et al*., 1997; Smith *et al*., 1997; Mitra *et al*., *2001;* 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*., 2004a; Singh *et al*., 2005; Cavalieri and Caporali 2008; Verdeguer *et al*., 2011, Jawahar *et al*., 2013). Among various aromatic plants, *Eucalyptus* species are very well known for their essential oil (EO) composed of a variety of volatile monoterpenes (Kohli and Singh, 1991). Among various species of *Eucalyptus*, *Eucalyptus tereticornis*is a good option for oil extraction because it is a tall evergreen plant with large leaf biomass which is easily available throughout the year and this plant species contain high amount of EO (Iqubal *et al*., 2003). Greater degree of biological activity like antioxident (Singh *et al*., 2009), pesticidal, nematicidal, herbicidal (Rassaeifar *et al*., 2013) and fungicidal properties (Arango *et al*., 2010) of its EO has also been reported. Keeping the above status in the mind, the present work was planned to check the phytotoxic potential of EO from the leaves of *E. tereticornis* against *E. crus-galli* L. with the objective of exploring its possible use as a bioherbicide.

MATERIAL AND METHODS

Collection and extraction of EO

The leaves of *E. tereticornis* were collected from the field near Central University of Punjab, Bathinda during March, 2011. Nearly 2 Kg of freshly collected leaves were chopped into small pieces and mixed well with 4L of water. The mixture was heated at 105 ºC for 2:30 hrs using Clevenger's apparatus and distilled oil was collected from the nozzle of the condenser. The oil thus obtained was passed through sodium sulfate bed to remove water vapours and stored at 4ºC till further use. In a similar manner, the oil was extracted five times and the average was noted aspercent oil yield (w/v basis).

Composition of essential oils

The composition and the identification of the EO were studied using Gas Chromatograph coupled with Mass Spectrophotometer(Shimadzu QP 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 was searched using reference book of Adams (2007). Kovats retention index of each component were determined by co-injection of oil with homologous series of *n*-alkanes (C_8-C_{32}) under exactly the same conditions.

Study of physiological parameters

For bioassay, the seeds of *E. crus-galli* L. were collected from the local fields of Bathinda, Punjab (India). All the chemicals purchased were AR grade from Sdfine Pvt. Ltd. and Loba Chem. Pvt. Ltd. India.

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* were dipped in distilled water for overnight. Further, seeds were sown in 15 cm diameter petri dishes lined with two layers of moistened Whatman no 1 filter paper. An aliquot of 0, 25, 50,100, and 250µg/mL of EO was applied on the inner side of the cover of petri dish. Immediately after the treatment, each petri dish with its cover was sealed with a piece of parafilm to reduce evaporation. A similar set up without EO served as control. All the experiments were done in triplicate and kept for one week at 25±2 °C in 12 hours dark and 12 hours light condition. The study was carried in terms of percent germination, measurement of root and shoot length and dry weight of 7-day-old seedlings. Total chlorophyll content was measured using method of Hiscox and Israesltam (1979). The cellular respiration or cell survival of treated and control seedlings were calculated using Steponkus and Lanphear (1967) method.

Biochemical Parameter analysis

Plant material was freed of pigment, fats and lipids by dissolving in acetone (for 72 hrs), followed by acetone: petroleum ether, 1:1 (for 24 hrs) and finally in petroleum ether (for 24 hrs). 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 protein content was estimated by the method of Lowry *et al.* (1951).

Calculation and statistics

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

RESULTS AND DISCUSSION

The essential oil (EO) extracted by Clevenger apparatus, after the extraction of oil the percent yield has been recorded near about 0.83%. Further the major constituents of EO were identified by using Gas Chromatography coupled with Mass Spectrophotometer. Result obtained from the GC-MS analysis show that the oil is a mixture of *monoterpenes*, sesquiterpenes, alcohols, ketons *etc*. Majority of the constituents were *monoterpenes* constituting approximately 72% of the oil. *α*-Pinene, 1, 8-Cineole and *β*-Pinene were the major *monoterpenes* constituting more than 50% of the oil. Apart from these, the other constituents in high concentration were *α*-Eudesmol and *β*-Eudesmol.

Table 1. Effect essential oil on root, shoot, germination and dry weight of weed *E. cruss-galli*

Concentration of oil (µg/ml)	Effect of essential oil in different Physiological Parameters			
	Root length (cm)	Shoot length (cm)	Dry weight (mg)	Germination in percentage
Control	8.6 ± 0.8^{a}	5.7 ± 0.7^{a}	10.2 ± 0.009^{a}	100^{a}
25	6.6 ± 0.2^{b}	5.1 ± 0.3^{a}	8.3 ± 0.004^{b}	89^{b}
50	5.6 ± 0.3^{c}	4.7 ± 0.7^{b}	8.1 ± 0.004^{b}	74^{c}
100	4.1 ± 0.1^{d}	4.1 ± 0.2^{b}	6.4 ± 0.003^{c}	56^{d}
250	1.4 ± 0.1^{e}	3.2 ± 0.4^{c}	5.2 ± 0.002^{d}	35^{e}

± SD where n=10 and different alphabets along each value represents significant differences over control at $P \leq 0.05$ applying Tukey's test

It was observed that the EO from *E. tereticornis* affected the seed germination of test plant *E. crus-galli* (Table 1). At lowest concentration (25µg/ml of EO), the germination of *E. crus-galli* was insignificant as compared to the control. Further, with increase in concentration of EO from 50-250 µg/mL, the germination was reduced significantly. At highest concentration 250µg/mL, reduction of almost 65% was observed. The results clearly indicate that the treatment of EO severely affects the weed germination

It was observed that the EO from *E. tereticornis* affected the development of shoot length of *E. crus-galli.* At lowest concentration of 25µg/ml of EO treatment, insignificant effect was observed on shoot length as compare to control. At higher concentrations100 to 250µg/mL of EO, the percent shoot length reduction increased to 29% and 44% respectively (Table 1). From table 1 it was observed that the root length reduced significantly in the treated seeds as compared to the control. In response to different treatment of EO the development of roots were different, in case of lowest concentration the reduction was 24% and at highest concentration it was 84%as compared to control.

The inhibitory effect of EO also reflected on the dry weight. At low concentration of 25µg/mL, the decrease in dry weight was lower *i.e.* approximately 19%, while on higher concentrations, the decrease in dry weight was higher up to 38% and 51% respectively (Table 1). The total chlorophyll content in test seeds exposed to EO was also drastically affected. In general, chlorophyll content decreased with increase in concentration of EO (Fig 1). The effect was even significant at lowest concentration of 25µg/mL. With increased EO concentration chlorophyll content declined drastically up to 80%. At 100 µg/ml concentration, there was a significant

reduction of approximately60% and at highest concentration of 250µg/ml, the chlorophyll content reduced up to 80%. In response to different concentration of EO (25 – 250µg/mL), cellular respiration in *E. crus-galli* seedlings reduced from 20to40%as compare to the control (Fig 1). In this regards previous works were also reported similar observation for cellular respiration, reduced the respiration process in *Parthenium hysterophorus*, *Triticuma estivum*, *Zea mays*, *Raphanus sativus*, *Cassia occidentalis*, *Amaranthus viridis* and *Echinochloa crus-galli.*

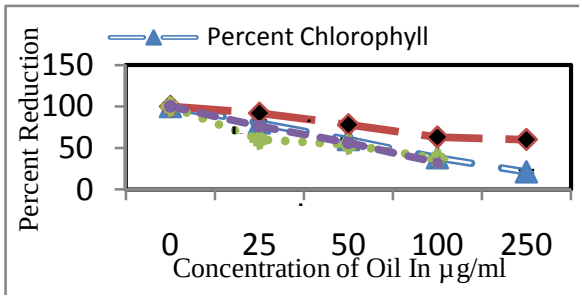

Fig 1. Effect of essential oil of *E. tereticornis* on the percent chlorophyll, respiration, carbohydrate and protein content.

The carbohydrate content in test seeds exposed to EO reduced with increase in concentration of oil (Fig.1). At 25µg/mL concentration, reduction was approximately 40% and at 50µg/mL concentration the reduction was insignificant *i.e* 42% as compared to 25µg/mL. But at highest concentration of 100 µg/mL, the content significantly reduced up to 60%. Like carbohydrate content, the protein content in test seeds exposed to EO also reduced with increase in concentration of oil (Fig.1). Likewise in carbohydrates, the reduction in protein content in *E. crus-galli* is less at lower concentration. At highest concentration of 100µg/mL, the protein content reduced approximately to 60%.

The seed germination, seedling length and dry weight of the test plant were significantly affected in response to vapors of EO from leaves of *E. tereticornis* plant. In general, a decrease was observed in tested parameters with increase in concentration of EO. Apparently the inhibition was directly proportional to the concentration of EO. Similar studies regarding phytotoxicity of EO of a number of plants and their pure components, earlier have also reported parallel results (Ponce *et al*., 2001; Singh *et al*., 2002; Angelini *et al*., 2003; Vokou, 2003; Batish *et al*., 2007; Kordali*et al*., 2008; Singh *et al*., 2006; Zanellato *et al*., 2009; Kaur *et al*., 2010; Chowhan *et al*., 2011). The mechanism of action of EO or its pure components include mitotic inhibition, inhibition of protein metabolism, changes in hormones, root tip inhibition, interfering with photosynthesis activity etc. (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).

It is evident from the results that treatment with *E. tereticornis* oil causes severe reduction in carbohydrate and protein content in shoots of test plant. The observation is similar to earlier reports that allelochemicals affect macromolecular content of other plants during growth inhibition (Zunino and Zygadlo, 2004; Nishida *et al*., 2005; Bainard *et al*., 2006; Singh *et al*., 2006, 2009; Mutlu *et al*., 2011). Parallel to carbohydrate content, the content of water soluble proteins decreased in response to EO from *E. tereticornis*. This decrease in protein content can be attributed to disturbed metabolic activities like photosynthesis and respiration of the seedlings (as already discussed) and another cause may be increased activity of protease enzyme as reported by number a of researchers (Singh *et al*., 2009; Bigham *et al*., 2010).This shows the phytotoxic potential of *E. tereticornis* EO against test weed *E. crus-galli* L and its possible use as bioherbicide in future.

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