

Original Research Article

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## Unravelling the Bioherbicidal Potential of *Eucalyptus* Species Aqueous Leaf Extract and Leaf Oil on Germination and Initial Growth Performance of Weed *Parthenium hysterophorus*

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### ABSTRACT

Trees, especially fast-growing multipurpose species, are becoming an integral part of intensive commercial agroforestry models introduced in recent times. Yet, the major hindrance for their productivity and survival is weed infestation with its toxic effects. They eventually decline the biodiversity by smothering native plants and disturbing their ecological niche. *Parthenium hysterophorus* invasion is a worldwide threat and acts as a potential menace in the biological phenomena of germination and growth, effecting plant biodiversity. Our study aimed to examine the outcome of aqueous leaf extract (ALE) and leaf oil (LO) of three exotic *Eucalyptus* species (*E. citriodora*, *E. tereticornis*, and *E. camaldulensis*) on germination and growth performance of *Parthenium hysterophorus* using two experiments. In the first experiment, we compared the inhibitory effect of aqueous leaf extract and leaf oil on seed germination of weed, while in the second experiment, we studied the different concentrations of aqueous leaf extract and leaf oil of promising *Eucalyptus* species from the first experiment on *Parthenium hysterophorus*. We measured the growth characteristics such as Germination percent, Speed of germination, Root length, Shoot length, Total seedling length, Seed vigor, and Phytotoxicity percent. The study highlights species *E. citriodora* found to be more promising for weed control and subsequently selected using different concentrations for the second experiment. Bioassay of *E. citriodora* oil at 5 ppm concentration was found to be more effective for weed suppression than aqueous extract concentrations. The study suggests an eco-friendly method to suppress the weed population and effective management in weed control.

#### Keywords

Phytotoxicity,  
Biodiversity,  
Parthenium,  
*Eucalyptus*

#### Article Info

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### Introduction

Trees perpetually contribute to the sustainability of food production and livelihood (Reed *et al.*, 2017). With the

increasing recognition of agroforestry as alternative land use, many scientists have focused their attention on trees. Most of these tree species are exotics and highly suitable due to their easy mode of maintenance, high

environment adaptations, fast growth, and high productive capacity (Gattoo, 2013). Among them, *Eucalyptus* has succeeded as a preferred species for agroforestry and farm forestry interventions throughout the country (Sachan 2006). *Eucalyptus* species (*E. citriodora*), Tasmanian blue gum (*E. globulus*), blue mallee (*E. polybractea*), and River red gum (*E. camaldulensis*) consists of tall, magnificent and evergreen trees with fragrant foliage rich in oil glands (Brooker and Kleinig 2006). Nevertheless, there is a continuing controversy about the ecological functions of the *Eucalyptus* tree. Its allelopathic effect on understory crops has also been reported by some studies (Sasikumar *et al.*, 2002; Kong *et al.*, 2019; Thiebout *et al.*, 2019). However, this effect may be exploited as an effective means to manage weeds, especially under agroforestry mode.

A weed possesses various toxicological effects on the ecosystem. Worldwide, a large amount of money is spent every year to control them. Weed infestation in agricultural fields results in huge economic losses and low-quality crop yields. In India, the total actual economic loss due to weed infestation was USD 11 billion in 10 major crops (Gharde *et al.*, 2018). A successful establishment of a weed in an ecosystem is attributed to its rapid growth rate, long-lived seeds, high reproductive potential, longer seed dispersal rate, competitiveness, and allelopathy (Gattoo *et al.*, 2013). Three Allelopathy ways have been mentioned for weed management, Firstly, by selecting desired crop variety, rotational use of allelopathy species, and lastly use of allelochemicals as natural herbicides (Rice, 1995; Farooq, 2020). The control of weeds can be achieved through several means, unfortunately, the use of synthetic herbicides may affect the environment and human health, and is also leading to increasing

resistance among many weed species. Globally, 2 million ton pesticides are being utilized and out of which herbicides contribution is 47.5% (Sharma *et al.*, 2019). Globally, there is a continuous up-gradation in herbicide-resistant weed species approx 262 thus, profusely expanded the use of more herbicides (Comont *et al.*, 2019; Heap 2020). Therefore, efforts to develop alternative means of weed control, which are not only eco-friendly but also cost-effective and bio efficacious are needed (Bhadoria 2010; Gnanavel 2015). In this direction, efforts to utilize allelopathy which is generally associated with the interaction between living plants, a significant role in plant-plant interaction, and natural plant products for effective weed management are being made (Singh *et al.*, 2003; Amist 2019). Growing environmental and public health concerns from the use of agricultural chemicals (pesticides) in agriculture have stimulated interest in the search for new and environmentally safe technologies for effective inhibition of weeds (Rice 1994; El-Metwally and El-Rokiek, 2019).

Allelopathy may be a successful tool to manage weed infestation in agricultural production if it can be exploited appropriately in a rotational cropping system (Khanh *et al.*, 2005). However, in the case of trees, it is difficult to apply the concept of rotation; therefore, enhancing weed suppression by trees itself through litterfall and isolation of natural plant products may be among the most feasible means of controlling weeds. The isolation and identification of allelochemicals responsible for weed suppression by trees may help understand the chemical interactions of trees and other plant communities.

Not all plants have allelopathic tendencies; a lot of tree-crop combinations are being practiced in the agroforestry system to form the suitable match. Many studies reported

negative interactions, thus, reduced growth and yield of the crop under trees due to phytotoxic interference viz., allelopathy (Singh 2001a). On the contrary, allelopathy could be beneficial in weed species by reducing weed emergence. The allelopathic effects of *Eucalyptus* have deleterious effects on another plant species by releasing Phenolic acids and volatile oils from the leaves, bark, and roots (Sasikumar *et al.*, 2001; El-Khawas and Shehata, 2005, Jawahar 2013; Puig 2018). *Eucalyptus* reported a combination of both positive and negative interactions on various crops and weed species (Schumann *et al.*, 1995; Romagni *et al.*, 2000; Sasikumar *et al.*, 2001). Allelopathic potential of *Eucalyptus* has been reported by few studies on chlorophyll content, respiratory activity, seedling length, and germination (Kohli *et al.*, 1998; Singh *et al.*, 2005; Hegab *et al.*, 2016; Wu *et al.*, 2019). Similarly, other species also documented deteriorating effects of *Eucalyptus* species on *Phalaris minor*, *Cassia occidentalis*, *Echinochloa crusgalli*, *Amaranthus hybridus*, *Portulaca oleracea*, *Cyperus rotundus*, and *Cynodon dactylon*, *Asphodelus tenuifolius*, *Brassica campestris*, *Triticum aestivum*, *Physalis hederifolia* and *Ipomoea species* (Batish *et al.*, 2004; Batish *et al.*, 2007; Khan and Marwat *et al.*, 2005; Babu and Kandasamy. 2008; Verdeguer *et al.*, 2009; Wu *et al.*, 2019). Besides *Eucalyptus*, species such as rice, sunflower, and sorghum were also studied for the inhibitory effect on *Parthenium* (Javaid *et al.*, 2006). Although the research on allelopathy in cropping systems has increased in the last two decades, the allelopathic influences of the comparative analysis of *Eucalyptus* species on weeds have been little investigated. The present investigation “Bioherbicidal potential of *Eucalyptus* aqueous leaf extract (ALE) and leaf oil (LO) on *Parthenium hysterophirum*” was carried out with the objectives to assess the inhibitory effect of ALE and LO of *Eucalyptus* species on germination

characteristics and growth performance of weed *Parthenium hysterophorus*.

## Materials and Methods

### Site description

The experiment was conducted during Rabi season in the laboratory conditions at Dr.Yashwant Singh Parmar University of Horticulture and Forestry, Himachal Pradesh (India). The center is located at 29<sup>0</sup> north latitude 30.92<sup>0</sup> N 77.12<sup>0</sup> E longitudes and altitude of 1256.40 meters above the mean sea level, which lies in the foothills of the Shivalik range of the Himalayas in the narrow strips.

### Plant material

25-year-old *Eucalyptus* species, *Eucalyptus citriodora* (lemon-scented gum) (E1), *Eucalyptus camaldulensis* (River Red Gum) (E2) and *Eucalyptus tereticornis* (Forest Red Gum) (E3) were used as the donor plant, collected from Dr.Y.S.P.University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh). The seeds of *P. hyterophorus* were procured from Norman E. Borlaug Crop Research Center, G. B. Pant University of Agriculture & Technology, Pantnagar. The seeds obtained were off last season in January, and the viability test was conducted during the experiment. The laboratory experiment was laid out in a factorial completely randomized design with three replications.

### Aqueous extract preparation

To evaluate the effect of a water-soluble extract of the *Eucalyptus* genus, fresh leaves of three *Eucalyptus* species were collected and washed thoroughly with tap water followed by distilled water to remove dust. After blotting dry with filter paper (Whatman

No. 1) 250 g of fresh leaves was ground in 500 ml of distilled water in a household blender for 10 minutes. The extract was then filtered through two layers of Whatman No. 1 filter paper and residue was washed thoroughly 2 to 3 times and made it up to 1 liter by the addition of distilled water, thus the stock solution of 100% aqueous extract was prepared. The stock solution was then stored in a refrigerator in a dark place in conical flasks until required. The extract was further diluted to 10, 25, 50, and 75 % extract concentration.

### **Extraction of volatile oil**

Oil was extracted by hydrodistillation using Clevenger's apparatus. Weighed fresh leaves of three Eucalyptus species viz.; *E. citriodora*, *E. camaldulensis* and *E. tereticornis* were collected. Freshly collected leaves of Eucalyptus species were weighed to 250 grams, chopped into pieces, and mixed with 1litre normal water at room temperature (210C) in a round bottom flask (2 liters) and boiled for 4 hours at 95 to 1000C temperature. The oil was collected from the nozzle of the condenser, stored in glass vials and dried under sodium sulfate, and stored at 40C in the freezer for bioassay studies. The oil was diluted to 0.25, 0.5, 1.0, 2.0, and 5.0 ppm at the time of application to seeds.

### **Measurements**

#### **Seed germination test**

Healthy and uniform size seeds of all test plants were selected and soaked in distilled water for 12 to 48 hours and treated with a small amount of thiram (quantity). The bottom of 15 cm diameter Petri dishes was covered with Whatman No. 1 filter paper. To test the inhibitory effect of leaf aqueous extract and oil, 10 ml of different concentrations and distilled water in control

were loaded on the inner side of the cover of the Petri dish. Twenty seeds of each test species were evenly placed in Petri dishes on a filter paper. Later on, two ml of extracts were subsequently added daily to keep the filter paper moistened. After spacing the seeds equally on the base, the Petri dish was immediately sealed with tape. Control was kept with distilled water. For each concentration, three replicates were maintained. All the Petri dishes were kept in an incubator at 200 C temperatures in dark.

### **Germination per cent**

The number of seeds germinated was counted daily up to the final day I. e.14 days for weed species or up to the time when the majority of seeds emerged and the normal seedlings were checked out. The seedlings with well developed, complete, proportionate and healthy essential structures were considered as normal seedlings and the seedlings with any one of the essential structures missing or badly damaged, weak or unbalanced development of essential structures and with any one of the essential structures showing diseased or decayed symptoms were counted as abnormal seedlings. Germination was observed daily according to the Association of Official Seed Analysts method (AOSA 1998). A seed was considered germinated when it had attained a 2 mm radicle length. Based on the above information seedlings were evaluated on the final day (disturbed or abnormal seeds were not counted) according to standard germination procedure and it was calculated as:

$$\text{Germination Percentage} = \frac{\text{No. of seeds produced normal seedlings}}{\text{No. of seeds set for germination}} \times 100$$

### **Speed of germination**

The number of seedlings emerging was counted daily from the day of planting the

seeds in the medium till the time germination is completed. Speed of germination also known as germination index was calculated with the method suggested by Association of Official Seed Analysts (AOSA 1983) using the following formula

$$\text{Speed of germination} = \frac{\text{No. of seedlings}}{\text{Day of first count}} + \frac{\text{No. of seedlings}}{\text{Day of final count}}$$

### Shoot/root/seedling length

The shoot and root length of seedlings were measured with the help of scale from tip to the base of the shoot and root at the final day after placing seeds in Petri dishes. The average shoot and root length were worked out by dividing total shoot and length (cm) with the number of seedlings. It was expressed in cm per seedling. The seedling length of the test plant species was obtained by the summation of root length and shoot length of seedlings and it was expressed in centimeters. It is the mean seedling length.

### Seed vigour index

Vigour index is the product of the germination percentage and mean seedling length and informed about the health of the plant. It is calculated by using the formula-

$$\text{Vigour index} = \text{Germination percent} \times \text{Mean seedling length.}$$

### Phytotoxicity per cent

The phytotoxic symptoms include shortened roots; discolored root tips; root raised from the paper; inhibition of root hairs development and root hairs bunched. The symptoms are more pronounced at an early stage of root growth. The phytotoxic symptoms are also evident in the plumular areas in the form of thickened or flattened plumules or, coleoptiles. The phytotoxicity

can be calculated as per the formula evolved by Surendra and Pota (1978).

$$\text{Phytotoxicity \%} = \frac{\text{Seedling length of control} - \text{Seedling length of treatment}}{\text{Seedling length of control}}$$

### Statistical Analysis

The experimental data obtained were subjected to statistical analysis one-way analysis of variance (ANOVA) using SPSS statistics 16 software, Tukey's *post hoc* test was used when variances remained homogeneous (Levene's test) and T3 Dunnett's *post hoc* test was employed if not, assuming equal variances. Differences were considered to be significant at  $p \leq 0.05$ .

### Results and Discussion

#### Germination percent

Different *Eucalyptus* species respond differently and significantly influenced the germination percent in both aqueous leaf extract (ALE) and leaf oil (LO) concentrations (Fig. 1). The study reported germination percent of *Parthenium hysterophorus* with maximum inhibitory effect of ~16.6 and 18.9 (*E. citriodora*; E1) followed by ~21.3 and 30.1 (*E. camaldulensis*; E2) and ~24.8 and 33.4 (*E. tereticornis*; E3) in both ALE and LO concentrations respectively (Fig. 1).

Further, bioassay of *E. citriodora* significantly impedes the germination of weed species. The inhibition effect increased with the increasing concentrations in both ALE and LO (Fig. 2). The germination percent was significantly and progressively reduced by maximum reduction in aqueous leaf extract and leaf oil concentration up to 100 % and 5 ppm respectively when compared to counterparts i.e. control (Fig. 2).



### Speed of germination

There was a profound reduction in the speed of germination with each *Eucalyptus* species of ALE and LO (Fig. 1). Significantly higher reduction in speed of germination was recorded in species E1 ~2.8 (LO) when compared to ~4.8 (ALE). Whereas, the accelerated speed was recorded in E3 by ~6.2 (LO) on comparison to ~5.5 (ALE) (Fig. 1). Subsequently, each increment in the concentration of ALE and LO of *Eucalyptus citriodora* decreased the speed of germination of *Parthenium hysterophorus* significantly (Fig. 3). In aqueous extract, maximum speed of germination was obtained at control, while 100 % leads to zero speed i.e. 100 percent inhibition (Fig. 3). Whereas, in LO concentrations, the maximum inhibition was recorded at 5.0 ppm (Fig. 3).

### Shoot and Root length

The inhibition potential of *Eucalyptus* species in shoots and root length of *Parthenium hysterophorus* was ranked as *E. citriodora* followed by *E. camaldulensis* and *E. tereticornis* (Table 1; A). Different concentrations of ALE and LO significantly suppressed the growth rate and the magnitude of inhibition was increased successively (Table 1; B). The application of ALE results maximum inhibition at 100 % with no root emergence, whereas 75% has inhibition of ~0.9 and ~0.24, in shoot and root part successively.

Similarly, LO concentration enhanced maximum suppression at 5 ppm concentrations with ~0.3 (Shoot) and ~0.17 (root) when compared to control ~2.5 (Shoot) and ~2.2 (root) (Table 1; B). Maximum deterioration in total seedling length was observed by the application of *E. citriodora* aqueous leaf extract followed by *E.*

*camaldulensis*. Lowest inhibition in the seedling length was observed in *E. tereticornis*. Decreasing trend in seedling length was found with increasing concentration from 0 to 100 % aqueous leaf extract and 5 ppm concentration of leaf oil. Interaction effect revealed the complete inhibition of seedling length of *P. hysterophorus* at 100 % aqueous leaf extract concentration in all the *Eucalyptus* species (Table 1; B).

### Seed vigour index

Seed vigour was significantly influenced by all *Eucalyptus* species as well as ALE and LO concentrations (Fig. 1 and 2). The seed vigour of *Parthenium hysterophorus* was significantly reduced to minimum in *E. citriodora* as compared to *E. camaldulensis* and *E. tereticornis* (Fig. 1). The seed vigour was significantly reduced with increase in concentration of aqueous leaf extract from 0 to 100 % (Fig. 3). At 75 % concentration up to 96 percent reduction was observed as compared to control. The increase concentration of leaf oil from 0 to 5 ppm significantly decreased seed vigour. From 0.25 to 5 ppm concentration the vigour was continuously reduced and reached to its minimum value at 5 ppm concentrations (Fig. 3).

### Phytotoxicity per cent

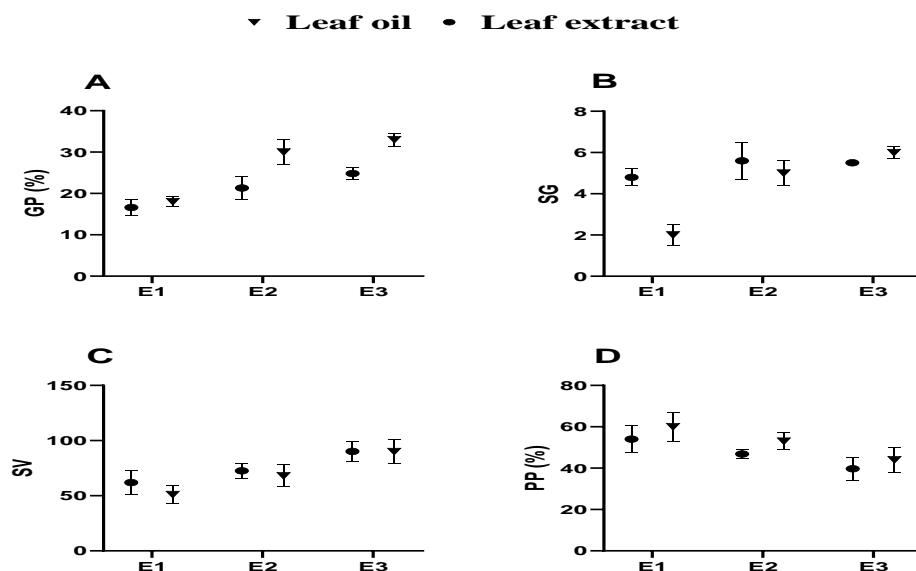
The phytotoxicity per cent was significantly influenced by each *Eucalyptus* species concentrations of aqueous leaf extract and leaf oil as represented (Fig. 1). Oil extract of *E. citriodora* has been proven more toxic than aqueous extract. Significantly higher phytotoxicity percent ~54 (LE); ~60 (LO) was recorded in *E. citriodora*, while minimum phytotoxicity percent ~39.7 (LE); ~44 (LO) was recorded under *E. tereticornis* (Fig. 1).

**Table.1** The response of Aqueous leaf extract (ALE) and Leaf oil (LO) of *Eucalyptus* species (A) and *E. citriodora* concentrations (B) on the average initial growth of *P. hysterothorus*

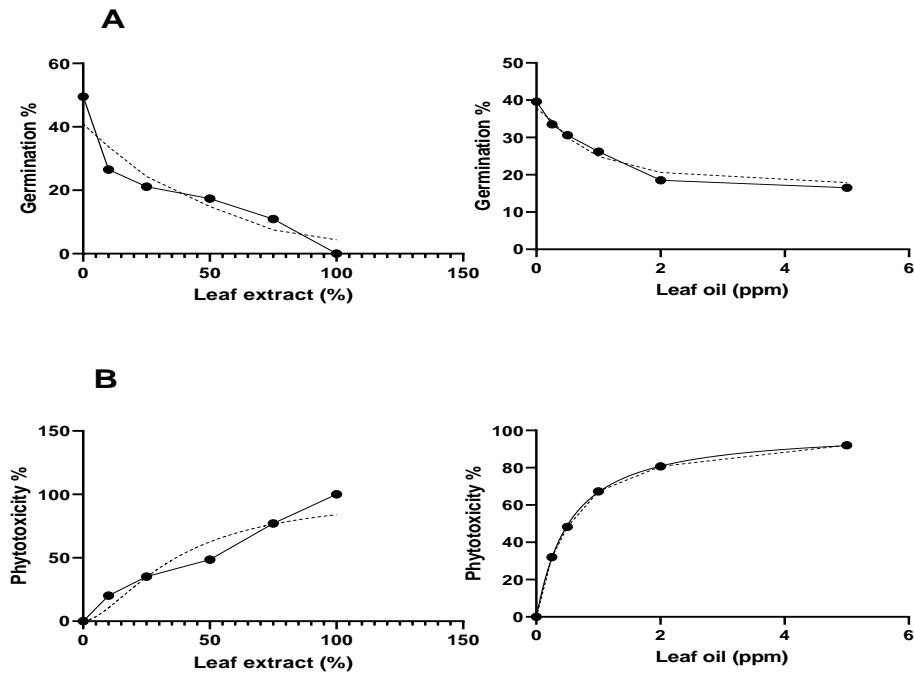
A		Aqueous leaf extract (ALE)			Leaf oil (LO)		
S. No.	<i>Eucalyptus</i> spp.	Root length (cm)	Shoot length (cm)	Total seedling length (cm)	Root length	Shoot length	Total seedling length
1.	<i>E. camaldulensis</i>	1.03	1.6	2.7	1.1	1.1	2.2
2.	<i>E. tereticornis</i>	1.08	1.9	3.0	1.3	1.4	2.7
3.	<i>E. citriodora</i>	0.97	1.30	2.3	0.83	0.89	1.7

B		Aqueous leaf extract (ALE)			Leaf oil (LO)			
S. No.	Concentrations (%)	Root length	Shoot length	Total seedling length	Concentrations (ppm)	Root length	Shoot length	Total seedling length
1.	Control	2.2	2.8	5.0	Control	2.2	2.5	4.7
2.	10	1.54	2.4	4.0	0.25	1.5	1.6	3.1
3.	25	1.22	2.0	3.2	0.50	1.1	1.3	2.4
4.	50	1.01	1.5	2.6	1.00	0.5	1	1.5
5.	75	0.24	0.9	1.2	2.00	0.4	0.5	0.9
6.	100	0.0	0.0	0.0	5.00	0.17	0.3	0.47

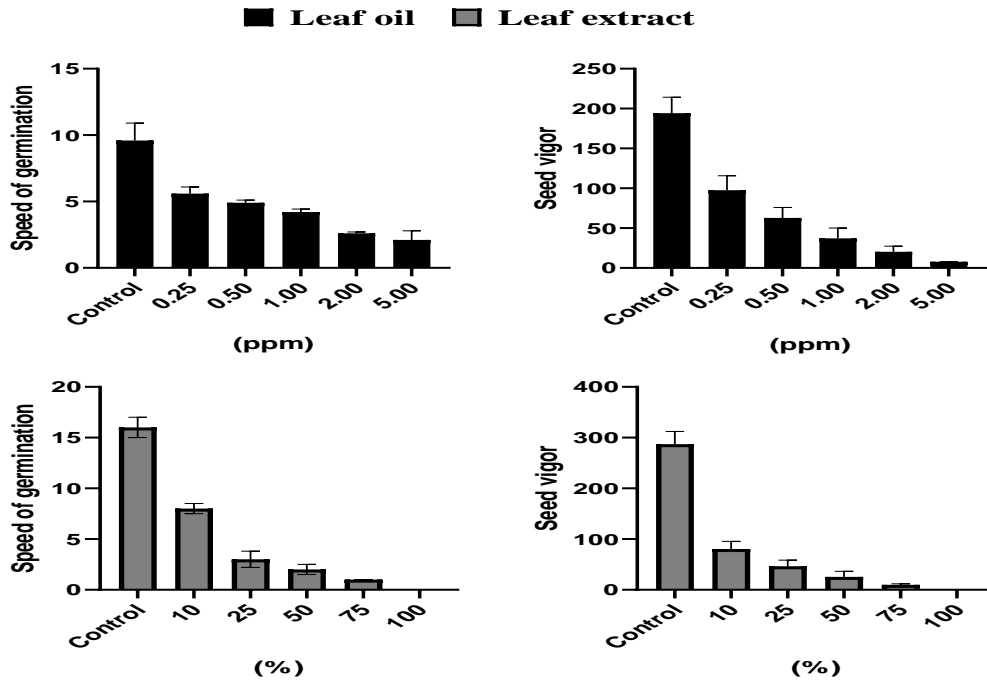
**Fig.1** Effect of leaf oil and aqueous leaf extract of three exotic *Eucalyptus* species (*E. citriodora* (E1), *E. camaldulensis* (E2) and *E. tereticornis* (E3) on A- Germination Percentage (GP); B- Speed of Germination (SG); C- Seed vigor (SV); D-Phytotoxicity Percentage (PP) of *P. hysterothorus* seeds



**Fig.2** A) Germination percent B) Phytotoxicity percent of *P. hysterophorus* seeds at different *E. citriodora* concentrations of aqueous leaf extract and leaf oil



**Fig.3** Effect of different concentrations of leaf oil and aqueous leaf extract of *E. citriodora* on Speed of Germination and Seed vigor of *P. hysterophorus* seeds





The increasing trend in phytotoxicity per cent was observed with every increase in the concentration of LE and LO from control to 100 % and 5 ppm respectively (Fig. 2). Control (distilled water) did not have any phytotoxic effect on *Parthenium hysterophorus*, whereas, successive increase in the concentration of aqueous extract from 10 to 100 % phytotoxicity percent enhanced. Significantly highest phytotoxicity 100 percent was recorded at 100 % aqueous leaf extract concentration against *P. hysterophorus*. Application of distilled water did not cause any phytotoxic effect on *Parthenium* but with an increase in the concentration of leaf oil from 0.25 to 5 ppm enhanced the phytotoxicity level. Significantly highest phytotoxicity 100 percent was recorded at 5 ppm concentrations against *Parthenium* weed (Fig. 2).

Allelochemicals also as nature's herbicides have several benefits over synthetic compounds. Natural compounds may have a novel structure due to the diversity of molecular structure. Natural products relatively have a short half-life and are biodegradable therefore, considered safe from environmental toxicology standpoint (Duke *et al.*, 2002). Among natural plant products, volatile essential oils and their constituents have attracted much attention because of their phytotoxicity (also providing allelopathic property) and relatively quicker degradation in the environment (Muller 1965; Kohli and Singh 1991; Romagni *et al.*, 2000; Singh *et al.*, 2002; Tworokoski, 2002; Ibanez and Blazquez 2018). Extensive commercial exploitation of natural derivatives has been reported in order to reduce the use synthetic pesticides (Masum *et al.*, 2016; Trezzi *et al.*, 2016; El-Mergawi and Al-Humaid 2019). Allelopathic agents influenced plant growth by affecting various physiological processes such as cell division and cell elongation, phytohormone induced growth, membrane

permeability, mineral uptake, stomatal uptake, photosynthesis and respiration (Rice 1994).

The allelochemicals specifically target the enzymes of plant metabolism and suppressed the growth and development mechanism. For instance, Terpenes present in the leaf part inhibit the germination process of seeds (Padhy 2000). The essential oil from *E. citriodora* was more toxic than *E. tereticornis* and it was attributed to the variability in their chemical constitution. A similar study was reported by Chaturvedi *et al.*, (2012) concluded that *Eucalyptus citriodora* is most toxic as compared to other *Eucalyptus* species examined on target weed species. Aqueous extract and oil concentrations proved highly effective against weed species where different employed treatments reduced the final germination by 100% (Isik *et al.*, 2016; Mekky *et al.*, 2019; Scavo *et al.*, 2019). Subsequently, Shafique *et al.*, (2005) found that the leaf extract of *M. indica* was highly suppressive against *P. hysterophorus*. Besides these, *Glycine max* and *Zea mays* also reduced the germination and seedling vigor in weeds *Bidens* and *Eleusine* species (Dhungana *et al.*, 2019).

Reduction in seedling growth may probably be due to the loss/ disruption of mitotic (Romagni *et al.*, 2000). The exact mechanism, by which seedlings length is affected by *Eucalyptus* oil, could be due to the inhibition of mitosis in the growing cells, as essential oils are reported to inhibit sprout growth in potato by killing meristematic cells. The seedling length of *parthenium* was reduced by 56% due to *Eucalyptus* oil (Singh *et al.*, 2005). Kohli *et al.*, (1998) have mentioned that the oil present in the leaves coins with the leaf diffusibility, rate, and stomatal aperture and eventually leads to wilting of the plant species, thereby suppressed the growth. This study has also been supported by reporting the n-hexane, acetone, and water-soluble

fractions inhibition shoot growth of lettuce with an increase in the concentration of the extracts (Ashrafi *et al.*, 2008). The Root length of weed species such as *Phalaris minor*, *P. hysterophorus* has reported to reduced by the application of *E. citriodora*, *E. globules extracts*, and *Alstonia species* (Kohli *et al.*, 1991; Javaid *et al.*, 2006).

Possessing great weed-suppressing ability, other studies have also been reported in essential oils of *E citriodora* phytotoxicity on weed species such as *Portulaca oleracea*, *Lolium multiflorum*, *Amaranthus viridis* and *Echinochloa crusgalli* (Ibanez and Blazquez 2019). Besides this, other *Eucalyptus* species; *E. camaldulensis* and *E. globulus* have also reported reducing the germination efficiency and phytotoxic effects on *Solanum lycopersicum*, *Lactuca sativa* and *Agrostis stolonifera* (Fikreyesus, 2011; Puij, 2018).

In conclusion the *E citriodora* oil has maximum inhibition impact with high phytotoxicity percentage than aqueous extract on weed *P. hysterophorus* and might be used as a potential species in weed management and as an alternative in weed control. There are indications of a possible role played by allelopathy in weed suppression by *Eucalyptus*; most of these studies have focused on the physical suppression of weeds. Thus, there is a need to critically analyze the differential response (if any) of allelochemicals secreted by *Eucalyptus* on weed species.

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