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Allelopathic effect of *Eucalyptus tereticornis* smith aqueous leaf extract on *Echinochloa crus-galli* (L.) P. Beauv

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Abstract
Weeds cause significant yield loss in crop plants. The use of herbicides is the economically viable technique for controlling weeds. However, the application of herbicides pollutes the soil, alter the microbial biota and exert a hazardous effect on human and animal health. The continuous use of specific herbicides for controlling weeds leads to the development of herbicide-resistant weeds. The use of natural metabolites from the plants exhibiting allelopathic potential is a novel strategy for sustainable weed management. *Eucalyptus tereticornis* is an excellent tree species with allelopathic properties. The study aimed to investigate different concentrations (5, 10 and 15%) of aqueous *Eucalyptus tereticornis* leaf extracts on the most noxious weed *Echinochloa crus-galli.* The dose-dependent inhibition was recorded in terms of germination percent, seedling growth and vigour. The aqueous leaf extract affected the emergence of the radicle. The inhibitory property of leaf extracts was due to the presence of allelopathic principles, which make *Eucalyptus tereticornis* as a promising candidate for obtaining bioherbicide.

Keywords: allelopathy, bioherbicide, echinochloa crus-galli, eucalyptus tereticornis, weed management

Introduction
Weeds are one of the important biotic factors in reducing the yield and quality of crops [1]**.** ICAR-Directorate of Weed Research (DWR) reported that India loses agricultural produce worth 11 billion dollars to weeds every year, which is more than that of 2017-18th union budgetary allocation for agriculture [2]**.** The most commonly adopted methods in weed management include the mechanical weeding of undesired plants and the application of herbicides [3]. Herbicide-resistant weeds, health effects, and environmental concerns are the significant challenges for the continuous use of herbicides to control weeds. In this context, the natural metabolites produced from plants referred to as allelochemicals have the potential to influence the weeds [4]. Allelochemicals are secondary metabolites synthesized in plants mostly from the shikimate and mevalonate pathways, which affect the germination and development of neighbouring plants [5, 6]. Allelochemicals show a similar mode of action in plants like herbicides, which provide the opportunity to explore as bioherbicides for controlling weeds. Several tree species, including *Eucalyptus sp.* exhibits allelopathic activity through the release of allelochemicals into the soil environment in the course of volatilization, leaching and root exudation, which in turn arrest the growth of weeds. Eucalyptus tree belongs to Myrtaceae family comprise of more than 900 species and subspecies, and are known for their insecticidal, larvicidal [7, 8], antimicrobial [7, 9], anti-inflammatory [10], antioxidant [11], and herbicidal activities [12, 13]. The herbicidal potential of *Eucalyptus sp.* has its significance in allelopathic research for weed management. The allelochemicals are eco-friendly and have no residual effect, which also has multiple sites of action with high specificity on target weeds. *Echinochloa crus-galli* is one of the most critical weeds, which competes with the field crops causing severe yield losses in crop plants. *Echinochloa crus-galli is an* annual and short-day plant possessing a C_4 photosynthetic pathway with several adaptive mechanisms for the survival in extremes of conditions. *Echinochloa crus-galli* infestation results in 21-71% of yield loss in rice crop, which is also included among the herbicide-resistant weeds [14, 15]. The present study was investigated to explore the phytotoxic effect of aqueous dry leaf extract of *Eucalyptus tereticornis* on the weed *Echniochloa crus-galli.*

Materials and Methods
Plant material and extraction of aqueous *Eucalyptus tereticornis* extract
Fresh leaves of *Eucalyptus tereticornis* were collected from five years old trees in the germplasm collection at Forest College & Research Institute, Mettupalayam (11°19'24.4"N 76°56'15.9"E). The aqueous extraction protocol was slightly modified from Arranz *et al*., 2010 [16].The fresh leaves of *Eucalyptus tereticornis* were washed thoroughly with tap water to remove the impurities adhered to the leaf surface. Then, the leaves were shade-dried and finely pulverized using a blender. The powder of *Eucalyptus tereticornis* leaf samples are placed in glass thimble and extracted using Soxhlet apparatus with water as a solvent. The extraction was refluxed until the completion of six cycles maintaining the temperature of the heating mantle at 100˚C.The aqueous crude leaf extract was allowed to cool at room temperature, which was filtered through Whatman No.1 and stored at 4 ˚C for characterization and assay studies.

Gas Chromatography/Mass spectrometry (GC-MS) analysis

GC-MS analysis of aqueous *Eucalyptus tereticornis* extract was carried out on a Thermo Scientific Trace GC Ultra with a TG-5MS capillary column (30 m x 0.25 mm x 0.25µm) equipped with DSQ II quadrupole mass spectrophotometer. Helium gas was used as a mobile phase with a maximum flow rate of 1.0 mL/min (pressure: 60-100 psi, 400-700 kPa).The rotary evaporator (Heidolph, Hei-VAP) was used to remove water from the crude extract of *Eucalyptus tereticornis* leaf samples. The contents were further freeze-dried to obliterate moisture. Then, the sample was dissolved in the n-hexane solvent, which was run entirely at a range of 50-650 m/z and the resulted mass peaks were processed and interpreted using the database of Xcalibur data processing system (NIST library MS version 2).

Bioassay studies of *Eucalyptus tereticornis* leaf extract on *Echinochloa crus-galli*

The bioassay study was carried out using *Echinochloa crus-galli* as a standard test species, and the seeds were collected from Wetland farm of Tamil Nadu Agricultural University, Coimbatore. Aqueous extracts of *Eucalyptus tereticornis* were diluted with sterile water to give final concentrations of 5, 10 and 15 per cent [17], while control was maintained using sterile water. Petri plates (9 cm diameter) and Whatman No. 1 paper discs were sterilized at 121˚C for twenty minutes. Seeds of *Echinochloa crus-galli* were surface sterilized using 1% sodium hypochlorite solution for 3 minutes and washed thoroughly with sterile water. Twenty-five seeds of the test species were placed in each Petri plates containing two layers of paper discs under laminar airflow chamber to avoid contamination during the germination. Various concentrations of leaf extracts of known volume (5 ml) were added carefully in the petri plate with disturbing the position of seeds. Treatments were arranged in a completely randomized design with five replications. The Petri plates were stored at room temperature in the growth chamber for six days.

Germinated seeds were counted at 24 h intervals during six days, while the length of shoot and root of *Echinochloa crus-galli* were measured seven days after sowing. Data were transformed to provide a degree of control for inferring results. The count of normal seedlings on 7the day of sowing was made and expressed as a percentage, while the vigour index was computed by adopting formula suggested by [18] and described as the whole number.

$$Vigour\ index = Germination\ percentage\ X\ Seedling\ length\ (cm)$$

Statistical analysis

The analytical mean data obtained during the experiment were statistically analyzed according to the analysis of variance technique (ANOVA) as well as the critical differences (CD) at 5% level of significance as described by [19]. The statistical analysis was performed by using AGRES statistical software.

Results and Discussion

Chemical composition of aqueous *Eucalyptus tereticornis* extract

The aqueous leaf extract of *Eucalyptus tereticornis* was analyzed through GC-MS, and the components were identified according to mass spectra. Thirty-three compounds were identified, representing 60.8 per cent of the ingredients (Table 1). The compounds include alkane hydrocarbon, fatty acid esters, aliphatic alkanes, alcohols, aromatic esters, saturated fatty acids, unsaturated fatty acids, ketones, steroids and terpenoids. The current study showed that aqueous *Eucalyptus tereticornis* leaf extract contains n-Hexadecanoic acid (6.77%) followed by 2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexene-1-one (5.91%), Squalene (5.00%), Octadecanoic acid (4.61%) and Octadecane, 3-ethyl-5-(2-ethylbutyl)- (4.34%). Parthipan *et al.,* (2015) reported n-hexadecanoic acid and Hexadecanoic acid, ethyl ester for its antioxidant, pesticidal, nematicidal, hypocholesterolemic, and hemolytic properties [20].Thunbergol is diterpene alcohol reported for its antimicrobial, anti-inflammatory, fungicidal and anticancerous properties. Squalene, a linear triterpenoid compound is known for its antioxidant, anticancerous and detoxifying properties [21]. Geranylisovalerate, a monoterpene ester identified in the analysis, was known for its natural fragrance. The compound dibutyl phthalate was earlier reported as a contaminant while the plastic material from the vials leaches out into the hexane solvent [22].The mass spectra of various compounds obtained during the analysis of GCMS is given in Figure 1.

Allelopathic activity of aqueous eucalyptus leaf extract

Germination and seedling growth of standard test species are widely used to understand the effect of allelopathy of donor plants. The aqueous leaf extracts of *Eucalyptus tereticornis* significantly inhibited the germination of test species *Echinochloa crus-galli.* In the present study, the aqueous leaf extract of 15 per cent showed the maximum inhibition on germination recording 52.8 per cent on germination illustrating the allelopathic effect on test species, while the control exhibited the germination of 97.6 per cent. The aqueous leaf extracts of 5 and 10 per cent registered germination of 83.2% and 69.6 %, respectively (Figure 2).The findings are in agreement with El-Ghit, (2016) [23] and Puig *et al.*,(2018) [24] who revealed that concentration of the *Eucalyptus leaf* extract determines the degree of germination inhibition. The germination and bioassay studies on *Echinochloa crus-galli* as a target species have been frequently investigated to access the negative allelopathic effect of the plant extracts and volatile oils [25, 26]. Dragoeva *et al*, (2015) [27] reported the morphological changes in the test species in response to the allelopathy due to the effect on a cellular or molecular level. Aqueous leaf extracts are employed in the study mimicking the precipitation process in nature, where water leaching along with water-soluble allelopathic compounds entering into the soil could prevent the germination of other plants. Vishwakarma *et al.,* (2015) [25] who reported the volatile oils from *Eucalyptus tereticornis* affected the germination of *Echinochloa crus-galli* weed species.

Aqueous eucalyptus leaf extracts affected the shoot and root length of *Echinochloa crus-galli* seedlings. Generally, germination is referred to as less sensitive to allelopathic substances than seedling growth, while allelopathic principles produce abnormal characteristics in seedlings. The results revealed that seeds treated with aqueous eucalyptus leaf of 15 per cent showed the maximum shoot inhibition (0.75 cm), followed by 10 and 5 per cent of leaf extracts, while the control exhibited the shoot length of 4.72 cm (Table 2). Root necrosis is the symptom exhibited in the receiver species associated with allelopathic effects. Higher concentrations of allelopathic

principles increase the number of abnormalities in the seedlings. Generally, the allelopathy results in the necrosis in the distal region of the roots, where the roots become thicker or exhibited signs of atrophy. The radicle region of the receiver species was much affected with blackened root tips. The control exhibited a radicle length of 6.55 cm, while seeds treated with the concentration of 15 percent exhibited the maximum radicle inhibition (0.04cm) followed by 10 and 5 per cent (Table 2). In this bioassay studies, the severe phytotoxicity confirmed the earlier report by Sale (2013) [28] wherein the leachate of dry *Eucalyptus tereticornis* leaves showed maximum inhibition and abnormalities in the radicle region of *Phaseolus vulgaris.* Morsi and Abdelmigid (2011) illustrated the effect of eucalyptus leaf extract in the mitotic phases during the cell division of barley seedlings. The record of more percentage of prophase cells at higher doses of eucalyptus leaf extract followed by the reduction of other mitotic phases, especially of metaphase. Results of cytogenetic analysis confirmed the disturbance of the mitotic process and induction of chromosome abnormalities in root-tip cells of barley. Sticky chromosomes and disturbance were recorded in prophase cell, while chromosome stickiness, spindle disturbances and vagrant chromosomes were observed in metaphase cells.

The seedling vigour was severely affected in all the treatments of aqueous eucalyptus leaf extracts. The highest seedling vigour (1102) was recorded in control, whereas seeds treated with eucalyptus leaf extract of 15 percent recorded vigour index of 43 indicating the sensitivity test species towards the extract (Table 2). Kaur *et al*, (2011) [30] reported similar results in *Amaranthus viridis* due to the treatment of the essential oil of *Eucalyptus tereticornis.* The dose-dependent decline of seedling vigour signifies the negative allelopathic effect of aqueous eucalyptus extracts.

Table 1: Chemical composition of aqueous *Eucalyptus tereticornis* extract

No	RT (min)	Name of the compound	Molecular weight (g/mol)	Peak area %
1	3.128	Decane	142.28	2.338
2	3.434	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	338.6	0.823
3	3.704	Hexadecanoic acid, ethyl ester (Palmitic acid, ethyl ester)	284.5	0.421
4	7.035	Dodecane, 2,6,11-trimethyl-	212.42	1.51
5	8.11	3-Cyclohexene-1-methanol, 2-hydroxy-à,à,4-trimethyl-	170.25	1.329
6	8.336	2-Butyl-2,7-octadien-1-ol	182.3	0.442
7	8.756	p-Mentha-1(7),2-dien-8-ol	152.23	0.986
8	8.811	3-Nonen-2-one, 3-ethyl-	162.28	1.061
9	9.921	(1S,2S,4S)-Trihydroxy-p-menthane	188.26	0.764
10	10.511	Disulfide, di-tert-dodecyl	402.8	0.579
11	12.162	2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	184.23	5.917
12	12.302	Eicosane, 7-hexyl	366.7	0.716
13	13.803	Heptadecane, 2,6,10,15-tetramethyl-	296.6	1.263
14	14.513	Thunbergol	290.5	0.568
15	14.713	Octadecane, 5,14-dibutyl-	366.7	0.512
16	15.088	2,5-Octadecadiynoic acid, methyl ester	290.4	3.917
17	15.568	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex 2-enone	222.28	0.807
18	16.634	17-Octadecenal	266.5	0.704
19	18.039	Nonadecane, 2-methyl	282.5	1.028
20	18.87	Dibutyl phthalate	278.34	2.758
21	19.12	n-Hexadecanoic acid	256.42	6.776
22	21.551	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	366.7	4.34
23	21.971	Hexadecane, 2,6,10,14-tetramethyl-	282.5	1.665
24	22.061	Docosanoic acid, 1,2,3-propanetriyl ester	1059.8	0.512
25	22.196	9,12-Octadecadienoic acid (Z,Z)-	280.4	1.036
26	22.291	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278.4	3.766
27	22.586	Phorbol 12,13-dihexanoate	560.7	2.212
28	22.791	Octadecanoic acid	284.5	4.614
29	22.976	n-Butyl ricinoleate	354.6	0.417
30	23.056	3-(4-Chlorophenyl)-1,2-diphenyl-2-propen-1-one	318.8	0.43
31	23.186	Spirostan-9-ol, 3-amino-, (3á,5à,25R)-	416.6	0.484
32	26.333	Geranylisovalerate	238.37	1.149
33	28.539	Squalene	410	4.99

Table 2: Effect of *Eucalyptus tereticornis* aqueous leaf extract on growth of *Echinochloa crus-galli*

Treatments	Shoot length(cm)	Root length(cm)	Vigour index
Control	4.74±0.87	6.55±1.54	1101.73
Aqueous leaf extract @ 5 per cent	1.37±0.16	0.43±0.16	149.68
Aqueous leaf extract @ 10 per cent	0.82±0.20	0.04±0.03	61.12
Aqueous leaf extract @ 15 per cent	0.75±0.18	0.036±0.02	42.72
SEd	0.29	0.37	43.28
CD (0.05)	0.62	0.78	91.75

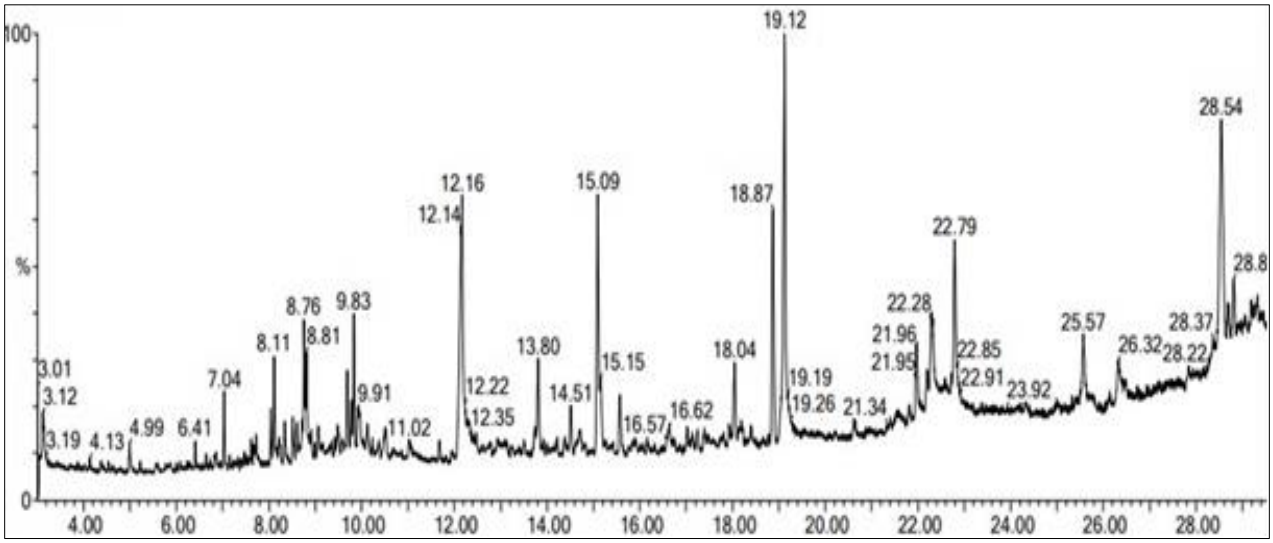
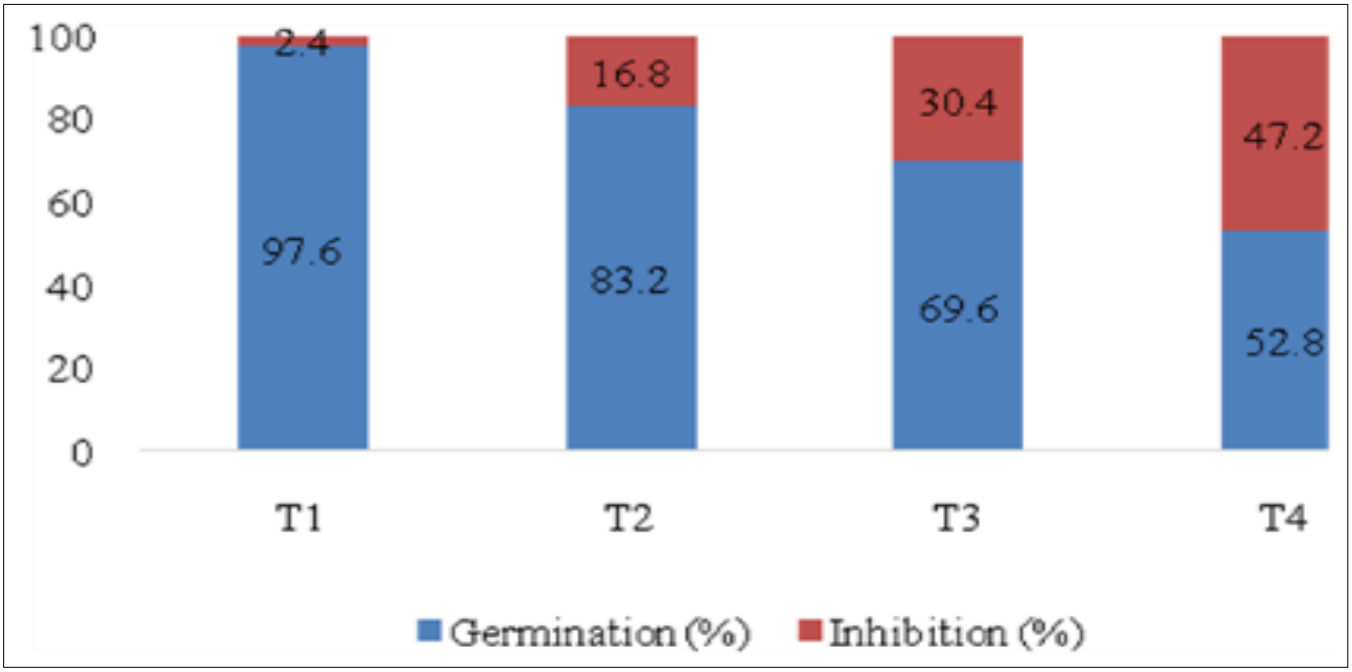
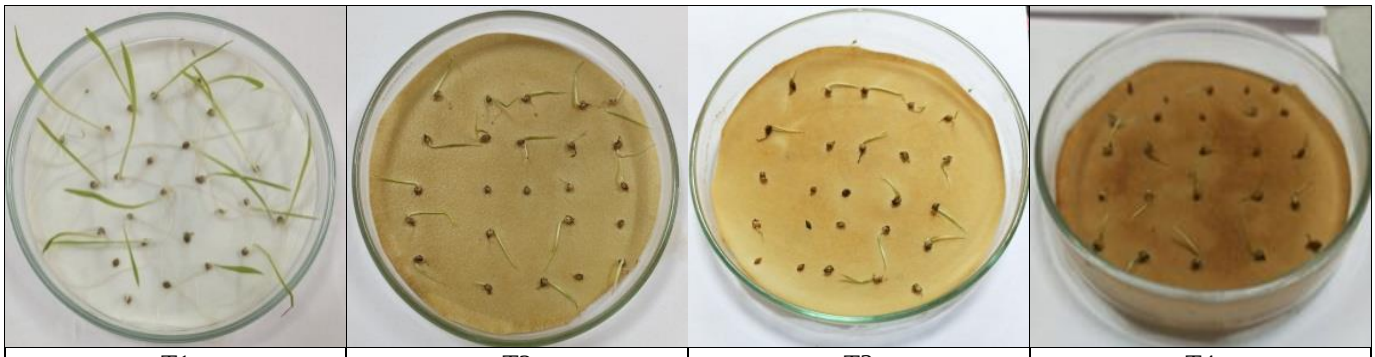


Fig 1: Mass spectra of aqueous *Eucalyptus tereticornis* leaf extract



Control (T1), Aqueous leaf extract @ 5 per cent (T2), Aqueous leaf extract @ 10 per cent (T3) and Aqueous leaf extract @ 15 per cent (T4)

Fig 2: Effect of different concentrations of eucalyptus leaf extract on germination of *Echinochloa crus-galli*



T1	T2	T3	T4

Control (T1), Aqueous leaf extract @ 5 per cent (T2), Aqueous leaf extract @ 10 per cent (T3) and Aqueous leaf extract @ 15 per cent (T4)

Fig 3: Bioassay studies to test *Eucalyptus tereticornis* aqueous -leaf extracts*on the germination of Echinochloa crus-galli*

Conclusion

Allelopathy, as a tool, will address the challenges of environmental pollution and herbicide resistance development due to the excess use of herbicides to manage weeds in agriculture. There is growing evidence for the use of allelochemicals for controlling weeds. Hence, the situation is pressing to identify the new molecules from the diverse plant kingdom. *Eucalyptus tereticornis* is a potent candidate containing allelopathic compounds responsible for the inhibition of weeds. The study was attempted to use aqueous leaf extracts of *Eucalyptus tereticornis* on the germination of *Echinochloa crus-galli.* The aqueous leaf extract of 15 per cent (w/v) significantly affected the germination and seedling vigour of *Echinochloa crus-galli.* The shelf life of allelopathic compounds is relatively less in the soil, which is the major challenge for scaling up into commercial scale. Hence, future research may be aimed at the isolation of specific compounds that are responsible for herbicidal action and encapsulation for improving shelf life of allelochemicals in the soil.

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