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Influence of Aqueous Leaf Extracts of *Eucalyptus tereticornis* Sm. On the Expression of Germination and Growth of Agricultural Seeds

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: This laboratory experiment was conducted to test the effect of allelopathy by *Eucalyptus tereticornis* aqueous leaf extracts on the germination and growth of Agricultural seeds i.e., *Arachis hypogaea, Cicer arietinum, Vigna radiata* and *Zea mays* as to test for suitable agroforestry system.

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Study Design: The experiment was conducted in a completely randomized block design.
Place and Duration of the Study: The experiment was conducted in the laboratory of Agroforestry and Soil Science, Department of Silviculture and Agroforestry, Forest College and Research Institute, Mulugu, Siddipet, Telangana, India.
Methodology: The aqueous leaf extracts of varied concentration [0 (Distilled water), 25, 50, 75 and 100% (m/v) %] prepared were tested against germination percent, relative germination percent, growth and relative allelopathic effect on the aforesaid agricultural seeds.
Results: The bioassay revealed that there was significant turndown in parameters tested. Control being the one relatively high performing, with increase in the concentrations of aqueous leaf extracts the germination rate, root length, shoot length, and dry weights reduced in all the other crops treatments tested.
Conclusion: The experiment attributes that leaching of allelochemicals from the leaves into the assay caused inhibitory effect on the seed germination and growth.

Keywords: Allelopathy; Eucalyptus tereticornis; Arachis hypogaea; Cicer arietinum; Vigna radiata; Zea mays; agroforestry; aqueous extracts.

1. INTRODUCTION

Allelopathy refers to the beneficial or harmful effects of one plant on another plant through the release of biochemicals (known as allelochemicals) through leachates, root exudation, volatilization and residue decomposition in both natural and agricultural systems [1]. These, allelopathic substances impede the growth and germination of neighbouring plant species [2]. However, allelopathic extracts can be used as herbicide for managing weeds [3]. Agroforestry, being a sustainable land management unit where woody perennials are deliberately grown with agricultural or horticultural components [4], it is important to devise such tree-crop Agroforestry systems, where effects of allelopathy are minimal.

Poor crop yields and poor growth of tree seedlings are often the result of inconsistent crop combinations. In fact, part of the problem lies in the selection of tree-food crop combinations and the inhibitory effect of some leaf exudates on adjacent crops. In research it is pointed out the need for research on allelopathy in various tree species used in agroforestry where allelochemicals released by cover crop trees are likely to affect food and forage crops [5]. Therefore, it seems important to check the allelopathic suitability of crops and trees before introduction into an agroforestry system [6].

Eucalyptus tereticornis and its clonal varieties are widely established plantations, which are commercially exploited for their pulp and also eucalyptus oil which has been used in variety of medications [7]. This tree species is therefore tested for its allelopathic impact on *Arachis hypogaea* L., *Cicer arietinum*, *Vigna radiata* and *Zea mays* because of their large-scale cropping in the study area i.e., maize accounting for 6.63 lakh ha, pulses for about 6.11 lakh ha and ground nut for about 1.89 lakh ha in the state of Telangana [8].

Identification of suitable tree-crop combination is essential to increase farm productivity and ecological sustainability of farmers. There are many arrays of crops intercropped with tree species but the pulses are considered suitable intercrops under agroforestry and mixed cropping systems to meet the dietary requirements and also to improve soil fertility and use as green fodder [9].

2. MATERIALS AND METHODOLOGY

The experiment was conducted between April-May 2023 in the laboratory of Department of Silviculture and Agroforestry, 2023 at Forest college and Research Institute, Mulugu, Siddipet District, Telangana.

2.1 Preparation of Aqueous Extracts

The leaves (a mixture of young, mature, old and dried litter leaves) of *Eucalyptus tereticornis* were collected from the plantation of Forest Research Centre, Mulugu. The leaves were air dried initially and then oven dried at 65^0C in hot air oven until constant dry weight is obtained [10]. The dried leaves were ground using grinder and used for experimentation. The aqueous extracts at different concentrations were prepared for the bioassays.

The ground powder of Eucalyptus leaves 200gm was added with 1 L of distilled water [11]. The solution was stirred and kept at room temperature for 24 hours. The filtrate was considered as 100% [12]. From this concentrate, following treatment concentrations were prepared using distilled water using (m/v) % i.e., mass of solute (aqueous extract) present in 100 ml of solution (Distilled water).

Table 1. Various concentrations of aqueous extract of *Eucalyptus tereticornis*

Treatment	Concentration
T1	25(m/v) % concentrated extract
T2	50(m/v) % concentrated extract
T3	75(m/v) % concentrated extract
T4	100(m/v) % concentrated extract
T5	Control

Each treatment is replicated five times such that the extract is put to test for its allelopathic effect in a Completely Randomized Design (CRD).

2.2 Petridish Bioassay

The seeds were pretreated for the removal of alien material through cleaning, grading, and sorting. Those pretreated seeds of *Arachis hypogaea* L., *Cicer arietinum*, *Vigna radiata,* and *Zea mays* were obtained from nearest market. Each Petri dish whose diameter is 90 cm was used for single replication. Ten seeds were placed on filter paper in the Petri dish. The 8 ml of aqueous extract was applied on first day and alternate day watering was done to keep the assay moist till the completion of the experiment [13]. The seed were considered as germinated when the length of radicle reached >1cm after emergence from the seed. The germination was estimated till 11 days after the start of the experiment. The growth of shoot and root in terms of root length, shoot length and biomass were recorded. To record the biomass, dry weight of root and shoot were recorded separately after them being treated in hot air oven at 60^0C for 48hrs [14]. Germination percent [15], Relative germination ratio [16], Relative allelopathic effect [15], root length, shoot length and biomass [17] were calculated as per standard procedure.

2.2.1 Germination percent

The germination percent was calculated as per reference 15

$$\text{Germination percent} = \frac{\text{Number of germinated seeds}}{\text{Number of sown seeds}} \times 100$$

2.2.2 Relative germination ratio (RGR)

The relative germination rate was calculated using reference 16

$$\text{Relative Germination ratio} = \frac{\text{Germination percent of tested crop}}{\text{Germination percent of control crop}} \times 100$$

2.2.3 Relative allelopathic effect (RAE)

The relative allelopathic effect was calculated as per reference 15

$$\text{Relative allelopathic effect} = \frac{O - C}{C} \times 100$$

Where;
O is the value of plant trait when test species is exposed to allelochemicals and C is mean of trait under control conditions.
A negative RAE value indicates an inhibitory effect, whereas a positive RAE value indicates a stimulatory effect.

3. RESULTS AND DISCUSSION

3.1 Seed Germination

Germination percent of agriculture seeds of the test crops showed significant variation in different treatments. With the increase in the concentration of aqueous extracts of *Eucalyptus tereticornis,* the germination rate of crops decreased (Table 2). Highest germination was observed in control followed by T2(80%) in *Arachis hypogaea*, T1(92%) in *Cicer arientum* and and T3(82%) in *Vigna radiata* and T2(16%) in *Zea mays* which are all significant in respective crops. Germination percent in T2 was higher than T1 in all crops except for *Vigna radiata* and *Cicer arietinum*. The mean relative germination percent was highest recorded in *Arachis hypogaea* followed by *Cicer arientum, Vigna radiata* and *Zea mays.* Within the tested seeds,highest RGR was reported in *Arachis hypogaea* in T2(88.89%) followed by T1(67.44%), T4(67.44%) and T3(44.19%) respectively. Similarly, in *Cicer arientum* highest RGR was observed in T1(93.88%) followed by T2(85.71%), T3(73.47%) and T4(73.47%), in *Vigna radiata* highest RGR was recorded in T3(93.18%) followed by T1(86.36%), T4(81.82%) and T2(79.55%), in *Zea mays* Highest RGR was recorded in T2(22.22%) followed by T1(13.89%), T3(13.89%) and T4(13.89%). Among all the agricultural test crops T2 has highest RGR in *Arachis hypogaea* and *Zea mays* whereas T1 in *Cicer arientum* and T3 in *Vigna radiata.*

Table 2. Effect on Germination percent and Relative Germination Rate of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*		*Cicer arietinum*		*Vigna radiata*		*Zea mays*	
	GP	RGR	GP	RGR	GP	RGR	GP	RGR
T1(25%)	58.00	67.44	92.00	93.88	76.00	86.36	10.00	13.89
T2(50%)	82.00	95.35	84.00	85.71	70.00	79.55	16.00	22.22
T3(75%)	38.00	44.19	72.00	73.47	82.00	93.18	10.00	13.89
T4(100%)	58.00	67.44	72.00	73.47	72.00	81.82	10.00	13.89
T5(Control)	86.00	100.00	98.00	100.00	88.00	100.00	72.00	100.00
C.D	18.41	21.41	16.91	17.26	9.21	10.46	11.88	16.50
SE(m)±	6.20	7.21	5.69	5.81	3.10	3.52	4.00	5.56

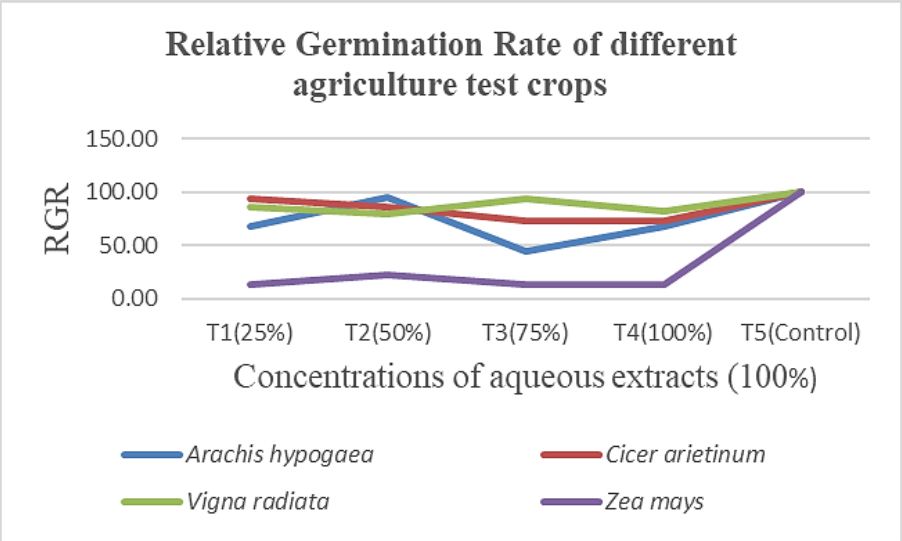

Fig. 1. Showing effect of various concentration of aqueous extracts of *Eucalyptus tereticornis* on Relative Germination rate

3.2 Shoot Length

The study resulted that there was significant variation in shoot length with the effect of change in concentration of treatments. Highest shoot length (25.45 cm) was recorded in *Zea mays* T5(control) and the least was recorded in T4(7.00 cm) in *Arachis hypogea.* In all the agricultural crop that were put to test viz., *Arachis hypogaea, Cicer arietinum, Vigna radiata* and *Zea mays* showed that with increase in the concentration of aqueous extracts the shoot length decreased significantly showing the negative effect on length of the shoot length.

Table 3. Effect on Shoot length(cm) of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*	*Cicer arietinum*	*Vigna radiata*	*Zea mays*
T1(25%)	9.32	18.77	15.26	22.42
T2(50%)	8.92	14.90	12.67	17.09
T3(75%)	7.28	12.53	11.63	15.61
T4(100%)	7.00	9.08	11.18	12.10
T5(Control)	10.04	15.71	15.47	25.45
C.D	0.87	4.16	1.77	5.32
SE(m)±	0.29	1.40	0.60	1.79

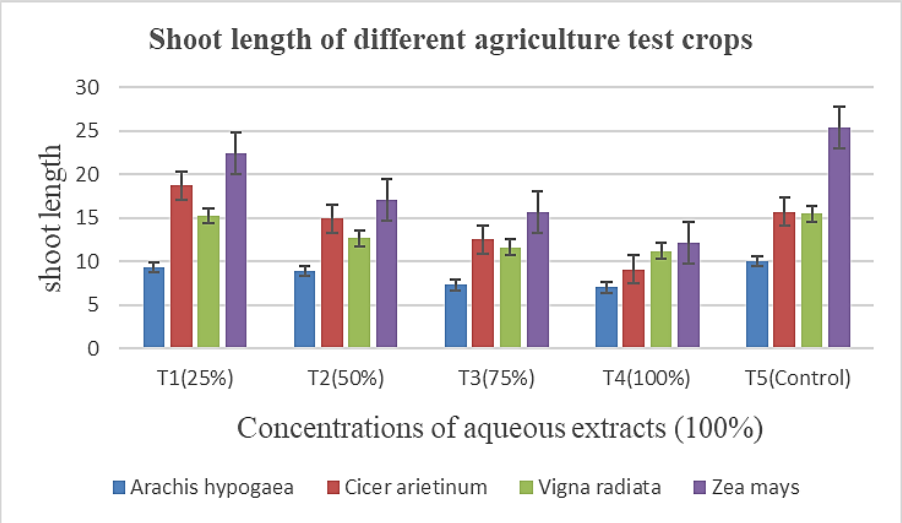

Fig. 2. Effect on Shoot length (cm) of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extract

3.3 Root Length

The experiment resulted that there was significant variation in different treatments. Usually with increase in the concentration of the treatment, the length of the root reported to decrease in all the agriculture crops put to study except for *Arachis hypogaea*. Among all the crops highest root length was observed in *Zea mays* T5(Control) (9.10 cm) and lowest was observed in *Arachis hypogaea* in T3 (0.91 cm). In *Arachis hypogaea* the highest root length was reported in control (2.12 cm) followed by T1(1.75 cm), T2(1.70 cm), T4(1.14 cm) and T3(0.91 cm). Similarly, in *Cicer arientum* T5(control) (8.36 cm) had highest root length followed by T1(8.22 cm), T2(7.92 cm), T3(4.25 cm), T4(2.96 cm). This trend is also observed in *Vigna radiata* and *Zea mays* with highest in control 2.74 cm and 9.10 cm respectively. In *Vigna radiata* after control it is followed by T1 (2.49 cm), T2(1.75 cm), T3(1.66 cm) and T4(1.50 cm) and in *Zea mays* it is T1(8.54 cm), T2(7.14 cm), T3(6.56 cm), T4(6.07 cm).

3.4 Shoot Fresh Weight (gm)

The aqueous extract of *Eucalyptus tereticornis* at different concentrations has shown a significant impact on the shoot fresh weights of agricultural crops put to study. The study discloses that with increase in the concentration of aqueous extract, the fresh weight of the crops decreased significantly except for *Arachis hypogaea* with anomaly at T1(2.64 gm) and T2(2.69 gm). The highest fresh weights are recorded in control treatment for all the crops. In Crops *Cicer arietinum, Vigna radiata* and *Zea mays* the fresh weights decreased with increase in the concentration of aqueous extract. Among all the crop highest shoot fresh weight was observed in *Arachis hypogaea* T5(Control) 3.06 gm) with lowest in *Cicer arietinum* in T3 (0.23 gm).

Table 4. Effect on Root length of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*	*Cicer arietinum*	*Vigna radiata*	*Zea mays*
T1(25%)	1.75	8.22	2.49	8.54
T2(50%)	1.70	7.92	1.75	7.14
T3(75%)	0.91	4.25	1.66	6.56
T4(100%)	1.14	2.96	1.50	6.07
T5(Control)	2.12	8.36	2.74	9.10
C.D	0.68	4.06	0.89	0.93
SE(m)±	0.23	1.37	0.30	0.31

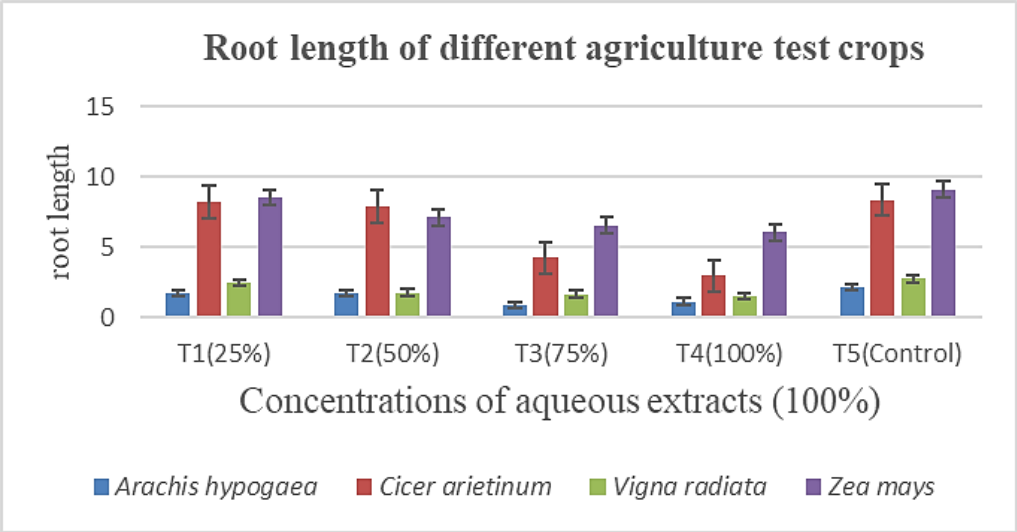

Fig. 3. Effect on Root length of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extract

Table 5. Effect on Shoot fresh weight (gm) of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*	*Cicer arietinum*	*Vigna radiata*	*Zea mays*
T1(25%)	2.64	1.15	0.89	0.90
T2(50%)	2.69	0.43	0.81	0.99
T3(75%)	0.29	0.23	0.76	0.45
T4(100%)	2.39	0.52	0.68	0.25
T5(Control)	3.06	1.02	1.51	1.14
C.D	1.31	0.49	0.25	0.51
SE(m)±	0.44	0.16	0.08	0.17

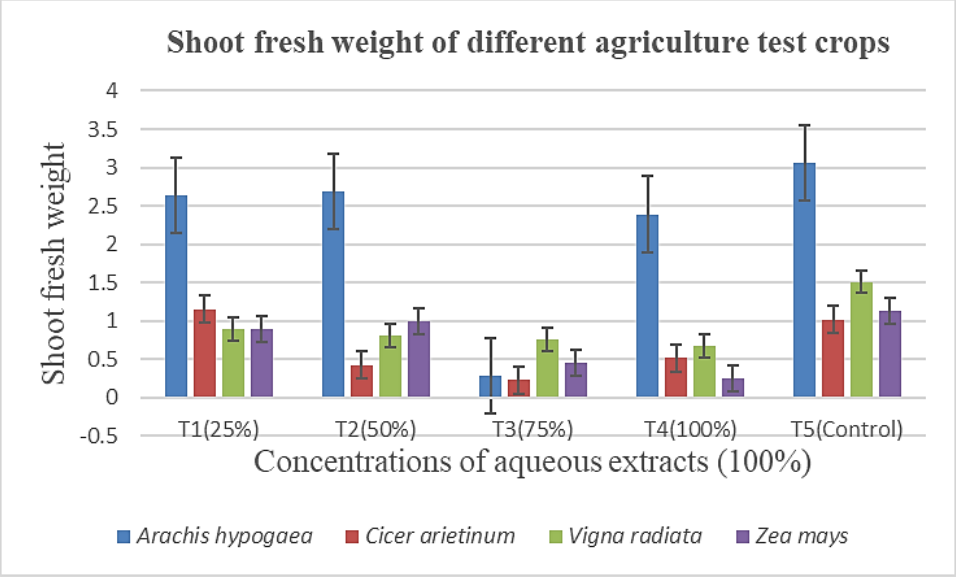

Fig. 4. Effect on shoot fresh weight of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

3.5 Root Fresh Weight (gm)

The aqueous extract of *Eucalyptus tereticornis* at different concentrations has shown a significant impact on the Root fresh weights of agricultural crops put to study. The study informed that highest fresh weights are observed in control in all the agricultural crops i.e., *Cicer arietinum, Vigna radiata* and *Zea mays* with exceptions in *Arachis hypogaea.* Similarly, in all the crops there was declining trend in weights with increasing concentration with exception in *Arachis hypogaea* (T1 with 0.34 gm, T2 with 0.59 gm, T3 with 0.09 gm and T4 with 0.36 gm ang T5 with 0.54 gm). Among all the crops highest root fresh weight was observed in *Zea mays* in T5(Control) (1.04 gm) with lowest in *Cicer arietinum* in T4 (0.19 gm).

3.6 Shoot Dry Weight (gm)

The effect of aqueous extracts of *Eucalyptus tereticornis* had shown significant variation on the dry weights of Agricultural crops considered for the study viz., *Arachis hypogaea, Cicer arientum*, *Vigna radiata* and *Zea mays.* The study revealed that with increase in the concentration of aqueous extract the weight of the crops decreased significantly. The highest weights are observed in Control followed by T1, T2, T3 and T4 in all the crops under the study. Among all the crops highest shoot dry weight was observed in *Cicer arietinum* in T5(Control) (1.03 gm) with lowest of 0.04 gm in T4 of *Vigna radiata.*

3.7 Root Dry Weight (gm)

The aqueous extract of *Eucalyptus tereticornis* at different concentrations has shown a significant impact on the Root Dry weights of agricultural crops put to study. The study informed that highest root weights are recorded in control followed by T1, T2, T3 and T4 in all the crops namely *Cicer arietinum, Vigna radiata* and *Zea mays* with minor exceptions in *Arachis hypogaea* (T1 with 0.18 gm and T2 with 0.18 gm). Among all the crops highest root dry weight was observed in T5(Control) (0.53 gm) of *Zea mays* with lowest in *Cicer arietinum* in T4 (0.03 gm).

Table 6. Effect on Root Fresh weight of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*	*Cicer arietinum*	*Vigna radiata*	*Zea mays*
T1(25%)	0.34	0.81	0.87	0.49
T2(50%)	0.59	0.51	0.60	0.46
T3(75%)	0.09	0.28	0.47	0.40
T4(100%)	0.36	0.19	0.33	0.33
T5(Control)	0.54	0.99	0.91	1.04
C.D	0.27	0.11	0.20	0.28
SE(m)±	0.09	0.04	0.07	0.09

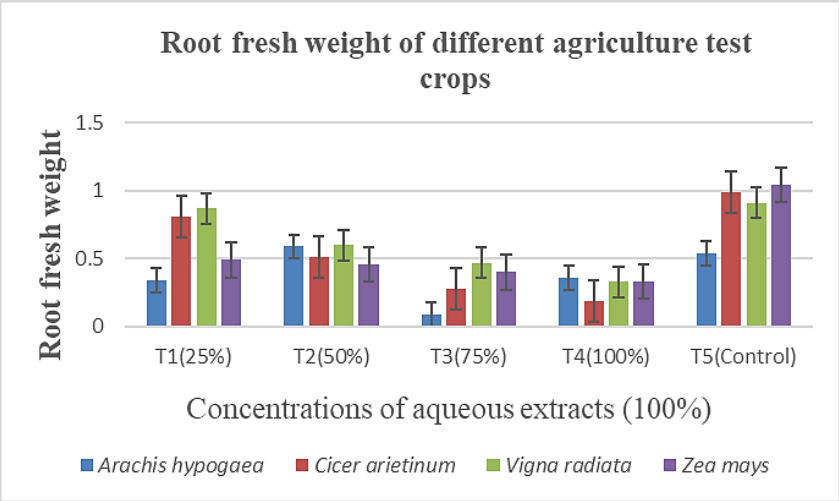

Fig. 5. Effect on Root Fresh weight (gm) of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Table 7. Effect on Shoot dry weight of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*	*Cicer arietinum*	*Vigna radiata*	*Zea mays*
T1(25%)	0.43	0.80	0.13	0.12
T2(50%)	0.25	0.57	0.07	0.10
T3(75%)	0.19	0.21	0.06	0.06
T4(100%)	0.05	0.08	0.04	0.05
T5(Control)	0.70	1.03	0.41	0.24
C.D	0.12	0.10	0.27	0.03
SE(m)±	0.04	0.04	0.09	0.01

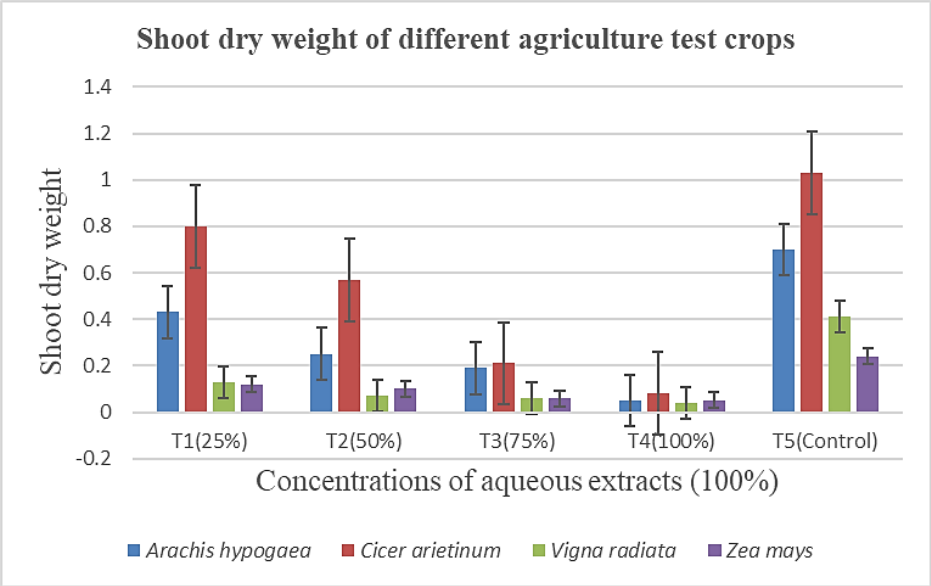

Fig. 6. Effect on Shoot dry weight of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Table 8. Effect on Root Dry weight of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

Concentrations of aqueous extracts (100%)	*Arachis hypogaea*	*Cicer arietinum*	*Vigna radiata*	*Zea mays*
T1(25%)	0.18	0.06	0.37	0.33
T2(50%)	0.18	0.05	0.31	0.30
T3(75%)	0.11	0.04	0.24	0.23
T4(100%)	0.05	0.03	0.04	0.18
T5(Control)	0.29	0.49	0.52	0.53
C.D	0.12	0.12	0.28	0.11
SE(m)±	0.04	0.04	0.09	0.04

3.8 Relative Allelopathic Effect

It is an index to determine the intensity of allelopathic effect on seed germination and seedling growth. The study revealed that there was an overall negative effect of allelochemicals leached into the bioassay of leaf extracts of *Eucalyptus tereticornis* on *Arachis hypogaea, Cicer arietinum, Vigna radiata* and *Zea mays.* The highest negative allelopathic affect was found on *Arachis hypogaea* at T3 i.e., 99.09% followed by T4(97.04%) in *Cicer arientum,* T4(98.5%) in *Vigna radiata* and T4(93.93%) in *Zea mays.*

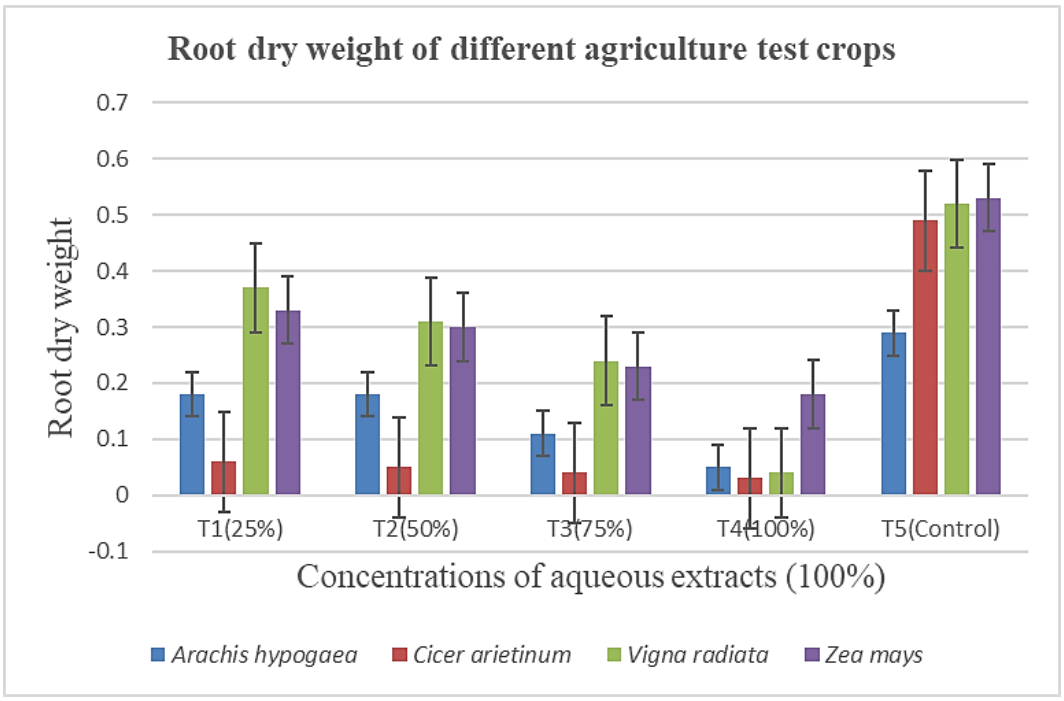

Fig. 7. Effect on Root Dry weight of different agriculture test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

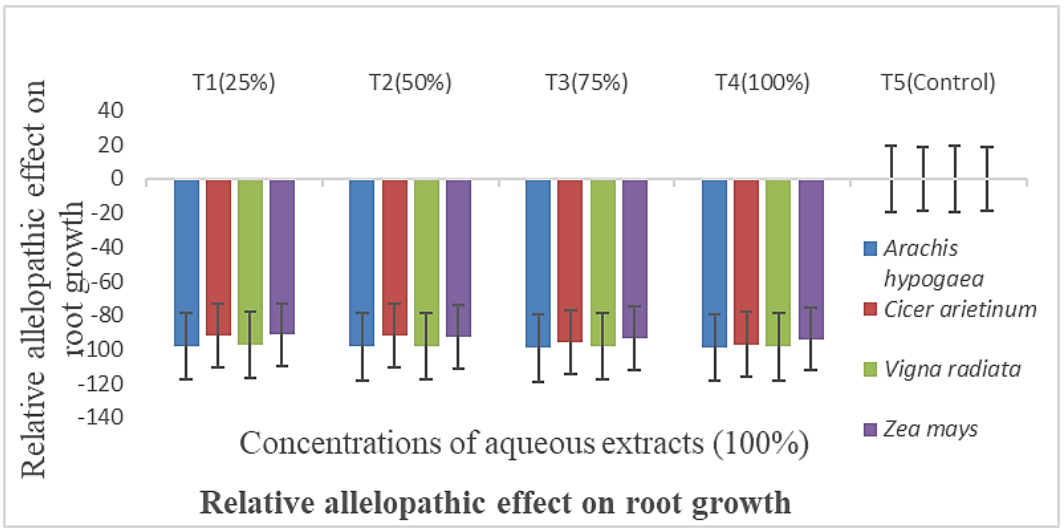

Fig. 8. Relative Allelopathic effect on the root length of agricultural test crops with application of different treatments of *Eucalyptus tereticornis* aqueous extracts

3.9 Discussion

The aforesaid results clearly sigh that there is a significant inhibitory impact of aqueous extracts of *Eucalyptus tereticornis* leaves on agricultural crops put to test. The results reported that there was negative effect on *Arachis hypogaea, Cicer arientum*, *Vigna radiata* and *Zea mays* with regard to germination percentage, root length, shoot length, root and shoot weights with increase in the concentration of leaf extract. Several researches such as in [18,19,20,21] also reported similar effect several *Eucalyptus species* on the agriculture crops under study and also the other crops. All *Eucalyptus species* contain leaf oil glands that are rich in essential oils, mainly terpenoids. These essential oils account for 1-5% of the fresh weight [22,23] and

also, leaves contain a wide range of phenolic chemicals [24,25]. It has long been known that many eucalyptus extracts have potent antibiotic activity [26]. It is found that eucalyptus leaf extracts (hybrid leaves) inhibited the germination of various edible plants [27].

The experiment revealed that there was significant effect of Eucalyptus leaf extract on biomass and root and shoot lengths of *Arachis hypogaea* with increase in the concentration i.e., similar to the experiments conducted by Soumare et al. [28] and Ghamini et al. [29] where there was a significant effect caused by allelochemicals of Eucalyptus leaf extract on the same. There is also a significant effect observed negatively on the germination, biomass, root and shoot lengths of *Vigna radiata, Cicer arientum* and *Zea mays* due to the allelochemicals released by leaf extracts of Eucalyptus. The same results as conducted in experiment were observed for *Vigna radiata* by Tripathi et al. [30] and Kumar [31], *Cicer arientum* by Samanta [32] and Ikram [33] and for *Zea mays* by Khan et al. [34] and Hegab et al. [35].

4. CONCLUSION

Eucalyptus species are one of the largely planted pulp yielding tree species accounting to meet industrial demands. Several Eucalyptus based agroforestry models [36,37] have been worked out to bring tree and agriculture crop under the same land management unit. But due to the allelopathic effect that is attributed to Eucalyptus there is a necessity to test the crop combination both at laboratory and field level. Considering the fact, laboratory test i.e., effect of foliar aqueous extracts of *Eucalyptus tereticornis* was conducted on seed of agricultural crops *Arachis hypogaea, Cicer arientum*, *Vigna radiata* and *Zea mays.* the study revealed a suppressive effect of extract on germination and growth of agriculture seeds put to test.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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