

# Assaying the allelopathic effects of *Eucalyptus camaldulensis* in a nursery bed incorporated with leaf litter

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**Abstract** Allelopathic effects of *Eucalyptus camaldulensis* Dehnh. were confirmed in Petri dish and pot experiments in our previous studies. However, the degree to which such effects under controlled experiments exist in more complex ecological settings remains to be tested. Thus, the present study was carried out by incorporating different proportions of ground litter of *E. camaldulensis* in soil. The growth of three agricultural crops: falen (*Vigna unguiculata* (L.) Walp.), chickpea (*Cicer arietinum* L.), and arhor (*Cajanus cajan* (L.) Millsp.), and two tree species, kala koroi (*Albizia procera* (Roxb.) Benth.) and ipil ipil (*Leucaena leucocephala* (Lam.) de Wit) were tested. There were inhibitory effects of leaf litter on germination, shoot and root growth, leaf number, and collar diameter as well as a reduction of nodulation by legume crops (25–80% reduction). The extent of the effects was dependent on the proportion of leaf litter, the species and the type of traits. In contrast to shoot growth, the effect on root growth was more severe. No effect on germination was found with the

agriculture crops while the two tree species showed reduced germination. The effect was greater in the presence of higher proportions of leaf litter mixed in soil while in some cases lower proportions stimulated growth. Not all species were suppressed; *A. procera*, *C. cajan*, *V. unguiculata* showed compatible growth while *C. arietinum* and *L. leucocephala* were found incompatible. This study provides evidence that *E. camaldulensis* has allelopathic potential under field conditions and a careful selection of associated crops in agroforestry systems is highly recommended.

**Keywords** Allelopathy · *Eucalyptus camaldulensis* · Inhibitory effects · Germination · Growth · Nodulation

## Introduction

Allelopathy is a well-known mechanism of plant-to-plant interaction exerting adverse effects on ecosystem structure and functions (Powell et al. 2013; Rice 1984; Si et al. 2013; Yuan et al. 2013). Association and dissociation patterns between certain species in an ecosystem may be governed by direct competition for necessary growth factors or through addition of allelopathic chemicals into the rhizosphere (Ashrafi et al. 2008; Bargali and Bargali 2000a, b; Einhelling 1996). The concept of allelopathy has received increased attention in the context of successful plant invasions over resource competition (Del Fabbro et al. 2014). The evidence of adverse allelopathic effects on germination, growth, development, and survival, nitrogen fixation and/or selective mycorrhizal fungi through the release of allelochemicals via leaching, litter decomposition, root exudation and/or direct volatilization has been shown in several studies (Abhilasha et al. 2008; Ahmed

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et al. 2004, 2007a, 2008; Hoque et al. 2003a, b; Inderjit 2005; Sun and He 2010; Uddin et al. 2007; Yang et al. 2007; Yuan et al. 2013). Consequently, there is a growing interest in studying allelopathy in suitable species combinations for commercial agroforestry (Ahmed et al. 2007b, 2008). *Eucalyptus* spp. are grown world-wide in association with agricultural crops, as windbreaks around orchards, and as avenue trees on farms (Ahmed et al. 2007b; Attiwill 1979; Bargali et al. 1992a, b, 1993; Bradstock 1981; Hasanuzzaman et al. 2004). Various species have been planted in many countries in preference over local species, owing largely to their versatility in adapting to a wide spectrum of climate and soil conditions, and in providing reasonable financial returns on a short rotation, i.e., 5–6 years. The wood of many species is suitable for a wide variety of uses (Davidson 1985). *Eucalyptus* spp. were introduced into Bangladesh in the nineteenth century, probably in the 1930's, in the eastern part of the country by tea planters as ornamental trees (Davidson and Das 1985). Later on, eucalypts were spread throughout the country in a haphazard manner by botanists, foresters, gardeners and tree planters (Hassan 1994). A total of 18,900 ha of plantations are under eucalyptus, 7% of the total plantation area in the country (Hossain and Hoque 2013). The plantations supply a vital demand for fuelwood, poles and posts for domestic use (Ahmed and Akhter 1995). Nevertheless, a controversy over the last few decades has developed as to whether *Eucalyptus* spp. are good or bad (Ahmed et al. 2008; Shiva and Bandyopadhyay 1983). They have been considered as rivals and competitors to endemic flora and environmentally unfriendly to Bangladesh (Ameen 1999), even though data is not enough to support this contention. There is a directive from the Government not to plant these species further (Letter of Ministry of Environment and Forests No. (Gen-3) 50/93/250 of dated 18.04.1995). In contrast, leading forestry and agroforestry experts and scientists have concluded that eucalypts may be suitable species for afforestation and reforestation in denuded areas and on marginal lands, in roadside plantations, and in agroforestry programs (Amin et al. 1995).

The allelopathy of eucalypts is considered the main cause for the reduction of biodiversity and the numbers of plantation forbs and graminoids, and reduced productivity of adjoining crops (Zhang et al. 2010). Our previous research supports the same phenomena of allelopathic effects on crops (Ahmed et al. 2004, 2007c, 2008). However, most of the evidence mainly relies on bioassays and pot experiments under controlled conditions. The major drawback of allelopathic research is the single application of extracts in most of the bioassay studies, which excludes abiotic and biotic manipulators of potential allelochemicals that would be encountered in the field (Ens et al. 2009). In

fact, various environmental factors including temperature, light, soil, precipitation, nutrients, water availability, and understory vegetation are important to consider as allelopathy greatly relies on them (Ahmed et al. 2008; Catherine et al. 2006, 2008; Khan et al. 1999). Past criticisms of allelopathic studies were based on the confusion between phytotoxicity and allelopathy and the failure to incorporate soil media, and the results were therefore regarded as lacking field relevance. Therefore, we hypothesized that a test of allelopathic effects could be different from a fully controlled situation if experiments were done in the soil where there is a chance of interaction between biotic and abiotic factors that would mimic nature. Thus, the present experiment was performed in the field to test whether the allelopathic effects of *Eucalyptus camaldulensis* shown earlier under controlled conditions (Ahmed et al. 2004, 2007c, 2008) are also detectable under ecologically more relevant soil-leaf litter mix conditions.

## Materials and methods

The research was carried out in the nursery of the Institute of Forestry and Environmental Sciences, Chittagong University in a randomized block design with five replications for each treatment. Leaf litter from 8-year-old *E. camaldulensis* was collected, air-dried and ground. The nursery bed was 2 m × 2 m separated by a 100 cm deep trench and leveled. All weeds and other debris were cleared. The bed was thoroughly prepared by adding 30 cm topsoil collected from a barren hillside and mixed with the ground leaf litter in the following proportions:

- T<sub>0</sub> = Seeds of crops grown in bed with barren hillside soil with no litter added (Control),
- T<sub>1</sub> = Seeds of crops grown in bed mixed with litter of 10 gm/m<sup>2</sup> (100 kg/ha),
- T<sub>2</sub> = Seeds of crops grown in bed mixed with litter of 50 gm/m<sup>2</sup> (500 kg/ha),
- T<sub>3</sub> = Seeds of crops grown in bed mixed with litter of 100 gm/m<sup>2</sup> (1000 kg/ha),
- T<sub>4</sub> = Seeds of crops grown in bed mixed with litter of 150 gm/m<sup>2</sup> (1500 kg/ha) and
- T<sub>5</sub> = Seeds of crops grown in bed mixed with litter of 200 gm/m<sup>2</sup> (2000 kg/ha)

The litter application rates varied from 100 to 2000 kg per hectare, which represented an average accumulation of 1028 kg/ha/year litter fall in an 18-year-mixed plantation (Haque 2013). After adding the litter mix soil, the bed was lightly irrigated and kept under a polythene sheet for a month. Seeds of the crops were sown after 3 months to allow the leaching and mixing of the released allelochemicals which were subjected to the exposure of other

biotic and abiotic interactions. The agricultural and tree crops selected were falen (*Vigna unguiculata*), chickpea (*Cicer arietinum*), arhor (*Cajanus cajan*), kala koroi (*Albizia procera*) and ipil ipil (*Leucaena leucocephala*). The agricultural crops were harvested after one and half months and the tree crops after two and half months.

The seed was considered germinated when the radicle emerged and germination was recorded daily up until the last radicle protruded. Watering and weeding were done when required. All growth parameters such as shoot and root length, collar diameter, lateral roots, nodule number and size, leaf, leaflet number, and root diameter were recorded. For the calculation of the degree of inhibitory effect on root and shoot elongation and collar diameter, the percentage compared to the control was calculated as per the formula developed by Surendra and Pota (1978). Duncan's Multiple Range Test (DMRT) using the SPSS package was used to analyze the data to a significance level of 1%.

## Results

### Germination

Germination of the agriculture and tree crops under different proportions of leaf litter mixture with the soil is shown in Table 1. All three crop species showed no effect even with the higher proportion of leaf litter compared to the controls. In contrast, germination of the two tree species was sensitive to the exposure to leaf litter and sensitivity progressively increased with the increase in litter. Of the two tree species, the performance of *L. leucocephala* was poorest (Table 1).

### Shoot and root length

Unlike germination, the response of shoot and root growth of all the seed material significantly varied with the amount

of leaf litter. The higher the percentage of leaf litter, the higher the inhibitory effect on all species, except the root growth of *C. arietinum* which was stimulated (Table 3) even though shoot growth was reduced (Table 2). Significant reduction in root growth of all other species was found with the T<sub>5</sub> treatment followed by a descending order of leaf litter component. Compared with the shoot growth, the effect on root growth was more severe. With the T<sub>5</sub> treatment, the reduction of primary root development of *C. cajan*, *V. unguiculata*, *A. procera* and *L. leucocephala* was 22.5, 56.93, 42.1, and 45.5%, respectively while that of shoot growth of those crops was 8.3, 40.61, 2.8 and 25.2%, respectively. Compared to controls, shoot growth of *A. procera* was less affected amongst the all species and was stimulated at the lower levels of litter (Table 2). However, root growth of *A. procera* was profoundly inhibited as was *V. unguiculata* and *L. leucocephala*, while root growth of *C. cajan* was less affected after *C. arietinum* (Table 3).

### Collar diameters

Collar diameters of seedlings with different proportions of leaf litter showed no statistically significant variations except for *L. leucocephala* (Table 4). Collar diameters were statistically different and suppressed with increasing of levels of litter. All other crops responded differently, showing both inhibited and stimulated growth with different treatments. The highest inhibitory effect (−32%) was found with *C. arietinum* seeds with T<sub>4</sub> treatment, while *A. procera* was the lowest (−1.1%) at the same treatment. The greatest stimulatory effect (+14.9%) was with *V. unguiculata* seeds with the T<sub>3</sub> treatment and the lowest (+0.7%) with *A. procera* and the T<sub>1</sub> treatment (Table 4).

### Leaf and nodule number

There was no consistent pattern of the effect of litter on leaf number of crops (Table 5). A lower proportion of litter showed stimulatory effects while the highest dosage

**Table 1** Germination percentage of crop/tree species to the application of proportions of leaf litter

Treatments	Germination of crop seeds				
	<i>C. arietinum</i>	<i>C. cajan</i>	<i>V. unguiculata</i>	<i>A. procera</i>	<i>L. leucocephala</i>
T <sub>0</sub>	48 a	58 a	93 a	73 a	45 b
T <sub>1</sub>	43 a	57 a	92 a	56 b	65 a
T <sub>2</sub>	45 a	58 a	93 a	70 a	42 b
T <sub>3</sub>	43 a	56 a	83 a	53 b	25 d
T <sub>4</sub>	45 a	56 a	77 a	52 b	30 d
T <sub>5</sub>	45 a	56 a	79 a	55 b	38 c

Values followed by the same letters are not significantly different according to Duncan Multiple Range Test (DMRT)

**Table 2** Shoot lengths (cm) with different proportions leaf litter

Treatments	Shoot length of crop seeds				
	<i>C. arietinum</i>	<i>C. cajan</i>	<i>V. unguiculata</i>	<i>A. procera</i>	<i>L. leucocephala</i>
T <sub>0</sub>	40.22 a	56.44 a	25.44 a	17.61 a	77.58 a
T <sub>1</sub>	32.33 a (-19.6)	54.78 a (-2.9)	20.00 bc (-21.4)	18.11 a (+2.8)	62.89 ab (-18.9)
T <sub>2</sub>	32.22 a(-19.9)	55.55 a (-1.6)	17.89 b (-29.7)	18.11 a (+2.8)	62.78 ab (-19.1)
T <sub>3</sub>	33.44 a (-16.8)	53 ba (-6.1)	21.67 b (-14.8)	15.5 b (-11.9)	59.56 b (-23.2)
T <sub>4</sub>	25.82 a (-35.8)	46.72 c (-17.2)	18.44 b (-27.5)	17.33 ab (-1.6)	61.72 ab (-20.4)
T <sub>5</sub>	28.55 a (-29.0)	51.78 ab (-8.3)	15.11 c (-40.6)	17.11 ab (-2.8)	58.0 b (-25.2)

Values followed by the same letters are not significantly different according to Duncan Multiple Range Test (DMRT), while values in parenthesis indicates stimulatory (+) or inhibitory (-) effects in comparison to control (T<sub>0</sub>) treatments

**Table 3** Root lengths (cm) of crop seeds with different proportions leaf litter

Treatments	Root lengths of crop seed				
	<i>C. arietinum</i>	<i>C. cajan</i>	<i>V. unguiculata</i>	<i>A. procera</i>	<i>L. leucocephala</i>
T <sub>0</sub>	10.74 ab	13.89 a	33.67 a	22.17 a	40.0 a
T <sub>1</sub>	12.22 a (+13.3)	11.0 b (-20.8)	16.89 b (-49.8)	13.11 cd (40.8)	27.78 bc (-30.5)
T <sub>2</sub>	10.19 b (-5.7)	11.94 b (-14.0)	16.33 bc (-51.5)	11.72 d (47.1)	30.72 bc (-23.2)
T <sub>3</sub>	9.64 b (-10.2)	12.0 b (-13.6)	15.83 bc (-52.9)	17.78 b (-19.8)	32.67 ab (-18.3)
T <sub>4</sub>	10.61 ab (-1.2)	12.0 b (-13.6)	17.5 b (-48.0)	14.27 c (-35.6)	22.0 c (-45.0)
T <sub>5</sub>	11.44 ab (+6.5)	10.77 b (-22.5)	14.5 c (-56.9)	12.83 cd (-42.1)	21.78 c (-45.5)

Values in the columns followed by the same letters are not significantly different according to Duncan Multiple Range Test (DMRT) while values in parenthesis indicates stimulatory (+) or inhibitory (-) effects in comparison to control (T<sub>0</sub>) treatments

**Table 4** Collar diameter of crop/tree species on the application of different proportions leaf litter

Treatments	Collar diameter (mm) of crops				
	<i>C. arietinum</i>	<i>C. cajan</i>	<i>V. unguiculata</i>	<i>A. procera</i>	<i>L. leucocephala</i>
T <sub>0</sub>	3.28 a	3.9 a	4.09 a	2.73 a	6.38 a
T <sub>1</sub>	3.61 a (+10.1)	3.4 a (-12.8)	3.71 a (-9.3)	2.75 a (+0.7)	5.91 ab (-7.4)
T <sub>2</sub>	3.35 a (+2.1)	3.83 a (-1.8)	4.31 a (+5.4)	2.57 a (-5.9)	5.69 b (-10.8)
T <sub>3</sub>	2.29 a (-30.2)	3.79 a (-2.8)	4.7 a (+14.9)	2.49 a (-8.8)	5.63 ab (-11.7)
T <sub>4</sub>	2.23 a (-32.0)	3.75 a (-3.8)	4.37 a (+6.8)	2.7 a (-1.1)	5.42 b (-15)
T <sub>5</sub>	2.81 a (-14.3)	3.83 a (-2.6)	4.07 a (-0.5)	2.3 a (-15.7)	4.54 c (-28.8)

Values followed by the same letters are not significantly different according to Duncan Multiple Range Test (DMRT) while values in parenthesis indicate the stimulatory (+) or inhibitory (-) effects

reduced the leaf number of all species except *V. unguiculata*. There was an inhibitory effect on nodulation of plants (Table 6), the trend increased with increasing of leaf litter. However, different plants responded differently to different treatments. *V. unguiculata* and *A. procera* had a higher number of nodules in control treatments, and then showed a significant uneven decreasing trend from treatment T<sub>1</sub> onwards. Nodulation of *C. arietinum* and *C. cajan* was

stimulated by the T<sub>1</sub> and T<sub>2</sub> treatments and then decreased. The average highest number of nodules (32.11) was recorded with *A. procera* under the control treatment while the lowest (0.33) was by *C. arietinum* with the T<sub>5</sub> treatment (Table 6). Nodulation in *L. leucocephala* was also observed but the nodules were distributed in such a way that they were impossible to count. In fact, nodule number in *L. leucocephala* was more than with any other seedlings.

**Table 5** Number of leaves of crops with application of different proportions litter

Treatments	Leaf numbers of crops				
	<i>C. arietinum</i>	<i>C. cajan</i>	<i>V. unguiculata</i>	<i>A. procera</i>	<i>L. leucocephala</i>
T <sub>0</sub>	50.33 b	22.57 a	18.67 ab	5.55 ab	22.83 b
T <sub>1</sub>	57.67 a	20.44 b	14.33 c	6.22 ab	30.33 a
T <sub>2</sub>	46.55 d	20.12 b	19.44 a	7.11 a	22.50 b
T <sub>3</sub>	47.88 cd	20.50 b	17.22 b	6.00 ab	18.33 c
T <sub>4</sub>	49.44 bc	20.22 b	18.55 ab	6.22 ab	24.17 b
T <sub>5</sub>	35 e	19.11 b	18.67 ab	4.33 b	14 d

Values in columns with the same letter are not significantly different according to Duncan Multiple Range Test (DMRT)

**Table 6** Nodule numbers of crops with the application of different proportions leaf litter

Treatments	Nodule number of crops			
	<i>C. arietinum</i>	<i>C. cajan</i>	<i>V. unguiculata</i>	<i>A. procera</i>
T <sub>0</sub>	2 ab	6.67 ab	14.22 a	32.11 a
T <sub>1</sub>	2.5 a	7.5 a	6.11 b	22.27 bc
T <sub>2</sub>	3 a	6.88 ab	6.44 b	23.44 c
T <sub>3</sub>	1.3 ab	5.1 bc	6.89 b	24.67 c
T <sub>4</sub>	1.3 ab	5.33 bc	6.44 b	24.55 c
T <sub>5</sub>	0.03 b	3.5 bc	4.78 b	16 d

Values in the columns followed by the same letter are not significantly different according to Duncan Multiple Range Test (DMRT)

## Discussion

This study has shown that soil mixed with *E. camaldulensis* leaf litter exerts negative effects on some tree species and agricultural crops, as demonstrated in vitro assays (Ahmed et al. 2004, 2007c, 2008). We assumed that the effect is not due to the slow decomposition of Eucalypt leaf litter (Bargali et al. 1993) but rather it could be attributed to the release of phytotoxic compounds. Many studies have documented that eucalypt litter could produce allelochemicals; for example, Zhang et al. (2010) identified 28 allelopathic compounds in *E. grandis* roots that are structurally similar to those reported to inhibit plant germination and growth (Djurđević et al. 2008; Nishimura et al. 1982; Rice 1984; Zhang et al. 2007). The negative impact of *Eucalyptus* species on undergrowth is evident from several comprehensive ecological studies; for instance, Bargali et al. (1992a, b) found a significant decrease in herb biomass and nutrient dynamics under a *Eucalyptus* plantation which progressively increased with plantation age from 2 to 8 years. We rule out the possible role of nutrient dynamics on the reduced growth of the crops, as the effect was compared to the control treatment where only soil was added.

The inhibitory effect of *Eucalyptus* leaf litter on nodulation supports earlier findings on the suppression of nitrification and/or nitrifiers in soils with a eucalypt cover (Adams and Attwill 1986; Jones and Richards 1977). Monoterpenes which are characteristic of eucalypt leaves were shown to inhibit nitrification in laboratory bioassays (Courtney et al. 1991; Ward et al. 1997; White 1986, 1991; Wood 1996). In agreement with previous results (Ahmed et al. 2004, 2007a, 2008; Ballester et al. 1982; Hoque et al. 2003a), we found the effects on the crops were concentration-dependent, increasing with an increase in litter, resulting in significantly smaller lengths of roots and shoots of target species compared to controls, with higher litter applications. This suggests that potential allelopathic effects could be more pronounced in areas with low or erratic rainfall due to relative higher concentrations of allelochemical substances (May and Ash 1990). In addition, relatively higher germination rates and early growth of the crop species were observed with lower quantities of litter, which was also corroborated by other studies (Ahmed et al. 2004, 2007c, 2008; Hong et al. 2004; Nektarios et al. 2005; Reigosa et al. 2000). The reasons are unclear and further research needs to be carried out. One explanation might be the small amount of nutrients brought by the litter partly contributes to the stimulatory effects (Khan et al. 1999). These effects were not consistent with all crops, nor were all the traits equally sensitive, as germination of the crops showed no effect while the tree species showed reduced germination. *C. arietinum* showed no effect on root growth but shoot growth was inhibited with the increase of litter applications.

The methodological approach of the present study is more robust than laboratory bioassays or other controlled experiments lacking complex ecological settings (Jandová et al. 2015). Methodological inadequacies that include the use of bioassays, insufficient controls and the lack of convincing field studies has raised the question of conclusions regarding the field relevance of allelopathy (Keeley 1988; Stowe 1979). The presence of bioactive



allelochemicals does not necessarily mean they have similar effects under both laboratory and field conditions. Exudates or leachates are much lower under natural conditions than in vitro because they are subject to absorption on soil particles as well as to chemical and microbial decomposition (Kaur et al. 2009; Lankau 2010). It is therefore equally important to carry out allelopathy tests adopting more ecologically relevant approaches along with in vitro bioassays. Our present study confirms the allelopathic effects of *Eucalyptus camaldulensis* in soil conditions where there was ample interaction between numerous biotic and abiotic factors, and thus representing the effect of more natural conditions. However, further research should be carried out using the soil of different ages of *Eucalyptus* plantations to see how reproducible the results are.

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