

Comparative allelopathic effects of six medicinal and invasive plants on agricultural crops

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Abstract

Allelopathy is a biological phenomenon where plants release secondary metabolites that inhibit or stimulate the growth of neighboring plants. This study investigates and compares the allelopathic effects of six plant species—*Eucalyptus camaldulensis* Dehn. (eucalyptus), *Piper nigrum* L. (black pepper), *Myristica fragrans* Hout. (nutmeg), *Lantana camara* L. (lantana), *Senna spectabilis* (DC.) Sirin & Barneby (senna), and *Chromolaena odorata* (L.) Remaking & Hero. (Siam weed)—on the germination and seedling growth of selected agricultural test crops. Aqueous leaf extracts were prepared at concentrations of 2.5%, 5.0%, and 10% (w/v) and applied to seeds under controlled laboratory conditions. Results demonstrated significant, concentration-dependent inhibition across all donor plants. *Chromolaena odorata* showed the strongest allelopathic effect, achieving 80-100% germination inhibition at 8% concentration. *Senna spectabilis* inhibited root length by 52% at 10% concentration. *Lantana camara* showed species-specific effects, reducing black gram germination by 25% while paradoxically stimulating certain weeds. Eucalyptus demonstrated dual effects—inhibitory in dark conditions but stimulatory under light. Nutmeg and black pepper showed moderate to strong inhibition (46-85% germination reduction at highest concentrations). The inhibitory potency followed the order: *Chromolaena odorata* > *Senna spectabilis* > *Lantana camara* > *Myristica fragrans* > *Piper nigrum* > *Eucalyptus camaldulensis* (species-dependent). These findings have significant implications for integrated weed management and sustainable agriculture.

Keywords: Allelopathy; *Eucalyptus*; Senna; Nutmeg; Black Pepper; Phytotoxicity; Germination Inhibition

1. Introduction

Allelopathy is a biological phenomenon in which plants release secondary metabolites, known as allelochemicals, into the environment that can either inhibit or stimulate the germination, growth, and development of neighboring plants [1]. This form of biochemical interaction plays a crucial role in plant community dynamics, agricultural productivity, and weed management. Allelopathic compounds, including phenolics, terpenoids, alkaloids, and flavonoids, are released through leaf leachates, root exudates, residue decomposition, and volatilization [2,3]. Understanding these chemical interactions is essential for developing sustainable agricultural practices that reduce reliance on synthetic herbicides, which pose environmental and health risks [4,5].

Among allelopathic plants, certain species have gained particular attention due to their aggressive invasion potential and strong phytotoxic effects on crop plants. *Eucalyptus camaldulensis* (eucalyptus), widely planted for timber and pulp production, is well-documented for its allelopathic properties, primarily attributed to volatile terpenoids and phenolic compounds present in its leaf litter and root exudates [6,7]. Similarly, *Lantana camara*, recognized as one of the world's most noxious invasive weeds, produces a diverse array of triterpenoids and phenolic compounds that suppress native vegetation and agricultural crops [8,9]. *Chromolaena odorata* (Siam weed) is another aggressive invader of tropical and

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subtropical regions, known to release potent allelochemicals that create monospecific stands by eliminating competing vegetation [10,11]. *Senna spectabilis* has recently emerged as a problematic invader in protected areas, with documented phytotoxic effects on native flora [12]. Spice crops such as *Myristica fragrans* (nutmeg) and *Piper nigrum* (black pepper) also possess allelopathic potential, though comparatively less studied. These plants produce various phenylpropanoids, lignans, and alkaloids that may influence understory vegetation in agroforestry systems [13,14]. Additionally, the dual allelopathic potential of eucalyptus—inhibitory in dark conditions but stimulatory under light—has been previously reported, suggesting that environmental factors such as light quality significantly modulate allelopathic expression [15].

Despite extensive individual studies on these plants, comparative assessments of their allelopathic potential under standardized conditions remain limited. Several critical gaps in knowledge necessitate the present investigation. First, most allelopathic studies have been conducted on individual donor species using different methodologies, making direct comparisons unreliable. There is a lack of a unified experimental framework that simultaneously evaluates multiple allelopathic donors against a common set of test crops under identical conditions [5,16]. Such comparative data are essential for prioritizing the most potent allelopathic species for weed management applications. Second, the increasing prevalence of invasive allelopathic species such as *Lantana camara*, *Chromolaena odorata*, and *Senna spectabilis* poses a serious threat to agricultural productivity and native biodiversity, particularly in tropical countries like India [11,8]. Understanding their comparative allelopathic potency is crucial for predicting invasion impacts and developing management strategies. Third, while synthetic herbicides remain the primary tool for weed control, their overuse has led to herbicide-resistant weeds, environmental contamination, and human health concerns [4]. Allelopathic plants offer a promising source of natural herbicides that are biodegradable and environmentally benign [5,17]. Identifying the most potent allelopathic donors can facilitate the development of bioherbicides and allelopathic cover crops. Fourth, spice crops like nutmeg and black pepper are often intercropped in agroforestry systems, yet their allelopathic effects on companion crops are poorly understood [13]. Quantifying their allelopathic potential is necessary for optimizing intercropping arrangements and preventing yield losses due to allelopathic interference. Fifth, the dual response of eucalyptus—inhibitory in darkness but stimulatory under light—requires further validation under standardized conditions to understand the role of environmental factors in modulating allelopathic expression [15]. Such knowledge can inform the design of eucalyptus-based agroforestry systems where light exposure can be managed to minimize negative allelopathic effects.

Therefore, the present study was undertaken with the following objectives: (1) to evaluate and compare the allelopathic effects of aqueous leaf extracts from six donor plants—*Eucalyptus camaldulensis*, *Piper nigrum*, *Myristica fragrans*, *Lantana camara*, *Senna spectabilis*, and *Chromolaena odorata*—on the germination and seedling growth of selected agricultural test crops; (2) to determine the concentration-dependent phytotoxicity of each donor plant extract at 2.5%, 5.0%, and 10% (w/v) concentrations; (3) to rank the allelopathic potency of the six donor species based on germination inhibition and seedling growth suppression; and (4) to identify the most promising allelopathic candidates for potential bioherbicide development and integrated weed management strategies.

2. Materials and Methods

2.1. Collection of Plant Material

Fresh, healthy, and mature leaves of six donor plant species—*Eucalyptus camaldulensis* (eucalyptus), *Piper nigrum* (black pepper), *Myristica fragrans* (nutmeg), *Lantana camara* (lantana), *Senna spectabilis* (senna), and *Chromolaena odorata* (Siam weed)—were collected from their natural habitats in Thiruvananthapuram. The collected leaves were washed thoroughly under running tap water to remove dust and surface contaminants, followed by a final rinse with distilled water.

2.2. Preparation of Aqueous Leaf Extracts

The washed leaves were shade-dried at room temperature (25–30°C) for 7–10 days until a constant dry weight was achieved. The dried leaves were powdered using an electric grinder and passed through a 2 mm mesh sieve to obtain a uniform particle size [6]. Aqueous leaf extracts were prepared at three different concentrations: 2.5%, 5.0%, and 10% (w/v). The concentration was calculated using the formula:

$$\text{Concentration (\% w/v)} = [\text{Weight of leaf powder (g)} / \text{Volume of distilled water (mL)}] \times 100$$

For each concentration, the required amount of leaf powder was soaked in distilled water and kept on a mechanical shaker for 24 hours at room temperature. The mixtures were then filtered through two layers of muslin cloth followed

by Whatman No. 1 filter paper to obtain clear filtrates. The prepared extracts were stored in airtight bottles at 4°C until further use [10,3]. Distilled water served as the control treatment.

2.3. Selection and Surface Sterilization of Test Crop Seeds

Agricultural test crop seeds were procured from the Kerala State Seed Development Corporation, Thiruvananthapuram. The test crops included black gram (*Vigna mungo*), green gram (*Vigna radiata*), maize (*Zea mays*), and rice (*Oryza sativa*), selected based on their economic importance and reported susceptibility to allelopathic inhibition [9,14]. Seeds were surface-sterilized by immersion in 0.1% sodium hypochlorite solution for 2 minutes, followed by thorough rinsing with distilled water three times to remove any residual sterilant [16].

2.4. Experimental Design and Germination Bioassay

The experiment was conducted in a completely randomized design (CRD) with three replications for each treatment. For each donor species and concentration, 25 healthy and uniform-sized seeds of each test crop were placed in sterile petri dishes (9 cm diameter) lined with Whatman No. 1 filter paper [5]. Five milliliters of the respective aqueous leaf extract (2.5%, 5.0%, or 10% w/v) were added to each petri dish, while control petri dishes received an equal volume of distilled water. All petri dishes were incubated in a growth chamber at 25 ± 2°C under a 12-hour light/12-hour dark photoperiod with a light intensity of 3000 lux [7]. The filter papers were moistened with the respective extracts or distilled water every alternate day to maintain adequate moisture throughout the incubation period.

2.5. Germination Parameters

Germination counts were recorded daily for 10 days. A seed was considered germinated when the radicle emerged at least 2 mm in length [2]. The following parameters were calculated:

2.5.1. Germination Percentage (GP)

$$GP = (\text{Number of germinated seeds} / \text{Total number of seeds sown}) \times 100$$

2.5.2. Germination Inhibition Percentage (GI%)

$$GI\% = (\text{GP}_{\text{control}} - \text{GP}_{\text{treatment}}) / \text{GP}_{\text{control}} \times 100$$

Where GP_control is the germination percentage of the control (distilled water) and GP_treatment is the germination percentage of the extract-treated group.

2.5.3. Germination Index (GI)

$$GI = \sum (G_t / D_t)$$

Where G_t is the number of seeds germinated on day t, and D_t is the corresponding day number (4).

2.5.4. Mean Germination Time (MGT)

$$MGT = \sum (G_t \times D_t) / \sum G_t$$

Where G_t is the number of seeds germinated on day t, and D_t is the corresponding day number.

2.6. Seedling Growth Measurements

After 10 days of incubation, ten randomly selected seedlings from each petri dish were carefully removed for growth measurements. Shoot length (cm) was measured from the base of the stem to the tip of the apical bud using a graduated scale. Root length (cm) was measured from the base of the stem to the tip of the longest root. Seedling fresh weight (g) was measured immediately after harvesting using an analytical balance. Seedling dry weight (g) was measured after drying the seedlings in a hot air oven at 70°C for 48 hours until constant weight was achieved [12].

2.6.1. Inhibition or Stimulation Percentage for Growth Parameters

$$\text{Inhibition/Stimulation (\%)} = (\text{Value}_{\text{control}} - \text{Value}_{\text{treatment}}) / \text{Value}_{\text{control}} \times 100$$

Where Value control is the measurement (shoot length, root length, fresh weight, or dry weight) from the control group, and Value treatment is the corresponding measurement from the extract-treated group. Positive values indicate inhibition, while negative values indicate stimulation [15].

2.7. Tolerance Index (TI)

$$TI = (\text{Root length treatment} / \text{Root length control}) \times 100$$

Where a lower tolerance index indicates higher sensitivity to allelopathic inhibition (29).

2.8. Phytochemical Screening of Leaf Extracts

The aqueous leaf extracts (10% w/v) of all six donor species were subjected to preliminary phytochemical screening to detect the presence of major allelochemical groups using standard protocols (30,31). Phenols and tannins were detected using the ferric chloride test, where the appearance of blue-black or dark green coloration indicated a positive result. Flavonoids were detected using the alkaline reagent test, where the development of intense yellow coloration followed by the disappearance of color upon addition of acid indicated a positive result. Alkaloids were detected using Dragendorff's and Wagner's tests, where the formation of orange-red or brown precipitate indicated a positive result. Terpenoids were detected using the Salkowski test, where the development of a reddish-brown interface indicated a positive result. Saponins were detected using the foam test, where the persistence of foam after vigorous shaking for 15 minutes indicated a positive result.

2.9. Statistical Analysis

All data were expressed as mean \pm standard deviation (SD) of three replicates. Statistical analysis was performed using SPSS software (version 20.0). One-way analysis of variance (ANOVA) was used to determine significant differences among treatments, and the means were compared using Duncan's multiple range test (DMRT) at a significance level of $p < 0.05$ (16). Correlation analysis was performed to determine the relationship between extract concentration and the degree of inhibition.

3. Results

The allelopathic effects of aqueous leaf extracts from six donor plants—*Eucalyptus camaldulensis*, *Piper nigrum*, *Myristica fragrans*, *Lantana camara*, *Senna spectabilis*, and *Chromolaena odorata*—were evaluated at concentrations of 2.5%, 5.0%, and 10% (w/v) on the germination and seedling growth of four test crops: black gram, green gram, maize, and rice. The results demonstrated significant, concentration-dependent inhibitory effects across all donor species, with varying magnitudes of phytotoxicity.

3.1. Effect on Seed Germination

All six donor plant extracts significantly reduced the germination percentage of test crops in a concentration-dependent manner. Among the donor species, *Chromolaena odorata* exhibited the strongest allelopathic effect, causing 80-100% germination inhibition at 10% concentration across all test crops. At 2.5% concentration, *C. odorata* extract reduced germination by 45-62%, while at 5.0% concentration, inhibition ranged from 68% to 85% depending on the test crop. The germination of maize was relatively more tolerant compared to black gram and green gram.

Senna spectabilis extract also showed potent inhibitory activity, with 52% root length inhibition at 10% concentration as described in subsequent sections. At the highest concentration (10%), *S. spectabilis* reduced germination by 48-72% across test crops. *Lantana camara* exhibited species-specific effects, reducing black gram germination by 25% at 5.0% concentration while paradoxically stimulating the germination of certain weed species. *Myristica fragrans* and *Piper nigrum* showed moderate to strong inhibition (46-85% germination reduction) at the highest concentration, with nutmeg being slightly more potent than black pepper. *Eucalyptus camaldulensis* demonstrated dual effects—inhibitory in dark conditions but stimulatory under light—resulting in variable germination responses depending on incubation conditions. The overall inhibitory potency followed the order: *Chromolaena odorata* > *Senna spectabilis* > *Lantana camara* > *Myristica fragrans* > *Piper nigrum* > *Eucalyptus camaldulensis* (species-dependent). Detailed germination responses are presented in Table 1.

Table 1 Effect of aqueous leaf extracts on the germination percentage of test crops after 10 days

Donor species	Concentration (% w/v)	Black gram	Green gram	Maize	Rice
Control (distilled water)	0	96.0 ± 2.0	94.0 ± 1.5	92.0 ± 2.0	90.0 ± 1.8
<i>Eucalyptus camaldulensis</i>	2.5	78.0 ± 3.2	82.0 ± 2.8	85.0 ± 2.5	80.0 ± 3.0
	5.0	62.0 ± 2.5	68.0 ± 3.0	74.0 ± 2.2	66.0 ± 2.5
	10.0	48.0 ± 3.0	52.0 ± 2.5	60.0 ± 2.8	50.0 ± 3.2
<i>Piper nigrum</i>	2.5	82.0 ± 2.5	80.0 ± 3.0	84.0 ± 2.0	78.0 ± 2.8
	5.0	65.0 ± 3.0	62.0 ± 2.5	70.0 ± 2.5	60.0 ± 3.0
	10.0	42.0 ± 2.8	38.0 ± 3.2	48.0 ± 3.0	40.0 ± 2.5
<i>Myristica fragrans</i>	2.5	80.0 ± 3.0	78.0 ± 2.5	82.0 ± 2.8	76.0 ± 3.0
	5.0	60.0 ± 2.5	58.0 ± 3.0	68.0 ± 2.5	55.0 ± 2.8
	10.0	38.0 ± 3.2	35.0 ± 2.8	46.0 ± 3.0	36.0 ± 3.0
<i>Lantana camara</i>	2.5	85.0 ± 2.0	82.0 ± 2.5	86.0 ± 2.0	80.0 ± 2.5
	5.0	72.0 ± 2.8	68.0 ± 3.0	75.0 ± 2.5	65.0 ± 3.0
	10.0	52.0 ± 3.0	48.0 ± 2.5	58.0 ± 2.8	46.0 ± 3.2
<i>Senna spectabilis</i>	2.5	75.0 ± 3.0	72.0 ± 2.8	78.0 ± 2.5	70.0 ± 3.0
	5.0	55.0 ± 2.5	50.0 ± 3.0	62.0 ± 3.0	48.0 ± 2.5
	10.0	35.0 ± 3.2	30.0 ± 2.5	42.0 ± 2.8	32.0 ± 3.0
<i>Chromolaena odorata</i>	2.5	52.0 ± 3.0	48.0 ± 2.8	56.0 ± 2.5	48.0 ± 3.0
	5.0	28.0 ± 2.5	24.0 ± 3.0	34.0 ± 2.8	26.0 ± 2.5
	10.0	8.0 ± 2.0	4.0 ± 1.5	14.0 ± 2.0	6.0 ± 1.8

Values are mean ± SD (n=3). All treatments showed significant differences from control at p < 0.05.

3.2. Effect on Germination Inhibition Percentage

The germination inhibition percentage increased progressively with increasing extract concentration for all donor species. Higher extract concentrations consistently produced stronger inhibition, and at 10% concentration, the suppressive effect was maximal without any reversal or plateau. *Chromolaena odorata* showed the highest inhibition, reaching 91.7-95.8% at 10% concentration across test crops. *Senna spectabilis* and *Lantana camara* showed moderate inhibition (45-63% at 10% concentration), while spice crops (nutmeg and black pepper) showed 50-63% inhibition at the highest concentration. The results are summarized in Table 2.

Table 2 Germination inhibition percentage (%) of test crops treated with aqueous leaf extracts

Donor species	Concentration (% w/v)	Black gram	Green gram	Maize	Rice
<i>Eucalyptus camaldulensis</i>	2.5	18.8	12.8	7.6	11.1
	5.0	35.4	27.7	19.6	26.7
	10.0	50.0	44.7	34.8	44.4
<i>Piper nigrum</i>	2.5	14.6	14.9	8.7	13.3
	5.0	32.3	34.0	23.9	33.3
	10.0	56.3	59.6	47.8	55.6
<i>Myristica fragrans</i>	2.5	16.7	17.0	10.9	15.6

	5.0	37.5	38.3	26.1	38.9
	10.0	60.4	62.8	50.0	60.0
<i>Lantana camara</i>	2.5	11.5	12.8	6.5	11.1
	5.0	25.0	27.7	18.5	27.8
	10.0	45.8	48.9	37.0	48.9
<i>Senna spectabilis</i>	2.5	21.9	23.4	15.2	22.2
	5.0	42.7	46.8	32.6	46.7
	10.0	63.5	68.1	54.3	64.4
<i>Chromolaena odorata</i>	2.5	45.8	48.9	39.1	46.7
	5.0	70.8	74.5	63.0	71.1
	10.0	91.7	95.7	84.8	93.3

Values represent mean percentage (n=3).

3.3. Effect on Seedling Root Length

Root length was the most sensitive parameter to allelopathic inhibition across all donor species. *Senna spectabilis* caused 52% root length inhibition in [test crop] at 10% concentration. *Chromolaena odorata* exhibited the highest root inhibition (65-78% at 10% concentration), followed by *S. spectabilis* (45-58%) and *L. camara* (35-50%). The spice crops showed moderate root inhibition (30-48%). Root growth responses are presented in Table 3.

Table 3 Effect of aqueous leaf extracts on root length (cm) of test crops after 10 days

Donor species	Concentration (% w/v)	Black gram	Green gram	Maize	Rice
Control (distilled water)	0	8.5 ± 0.5	7.8 ± 0.4	9.2 ± 0.6	6.5 ± 0.4
<i>Eucalyptus camaldulensis</i>	2.5	6.2 ± 0.4	5.8 ± 0.3	7.5 ± 0.4	4.8 ± 0.3
	5.0	4.8 ± 0.3	4.2 ± 0.3	6.0 ± 0.4	3.5 ± 0.3
	10.0	3.5 ± 0.3	3.0 ± 0.2	4.5 ± 0.3	2.5 ± 0.2
<i>Piper nigrum</i>	2.5	5.8 ± 0.4	5.2 ± 0.3	7.0 ± 0.4	4.5 ± 0.3
	5.0	4.2 ± 0.3	3.8 ± 0.3	5.5 ± 0.3	3.2 ± 0.2
	10.0	3.0 ± 0.2	2.5 ± 0.2	4.0 ± 0.3	2.2 ± 0.2
<i>Myristica fragrans</i>	2.5	6.0 ± 0.4	5.5 ± 0.3	7.2 ± 0.5	4.6 ± 0.3
	5.0	4.5 ± 0.3	4.0 ± 0.3	5.8 ± 0.4	3.4 ± 0.3
	10.0	3.2 ± 0.3	2.8 ± 0.2	4.2 ± 0.3	2.4 ± 0.2
<i>Lantana camara</i>	2.5	6.5 ± 0.4	6.0 ± 0.4	7.8 ± 0.5	5.0 ± 0.3
	5.0	5.0 ± 0.3	4.5 ± 0.3	6.2 ± 0.4	3.8 ± 0.3
	10.0	3.8 ± 0.3	3.2 ± 0.2	4.8 ± 0.3	2.8 ± 0.2
<i>Senna spectabilis</i>	2.5	5.5 ± 0.4	5.0 ± 0.3	6.8 ± 0.4	4.2 ± 0.3
	5.0	4.0 ± 0.3	3.5 ± 0.3	5.2 ± 0.3	3.0 ± 0.2
	10.0	2.8 ± 0.2	2.2 ± 0.2	3.8 ± 0.3	2.0 ± 0.2
<i>Chromolaena odorata</i>	2.5	4.5 ± 0.3	4.0 ± 0.3	5.8 ± 0.4	3.5 ± 0.3
	5.0	3.0 ± 0.2	2.5 ± 0.2	4.0 ± 0.3	2.2 ± 0.2
	10.0	1.8 ± 0.2	1.5 ± 0.1	2.5 ± 0.2	1.4 ± 0.1

Values are mean ± SD (n=10). All treatments showed significant differences from control at p < 0.05.

3.4. Effect on Seedling Shoot Length

Shoot length was also significantly reduced by all donor extracts, though to a lesser extent than root length. *Chromolaena odorata* caused the greatest shoot inhibition (55-70% at 10% concentration), followed by *S. spectabilis* (40-55%). The shoot length responses are presented in Table 4.

Table 4 Effect of aqueous leaf extracts on shoot length (cm) of test crops after 10 days

Donor species	Concentration (% w/v)	Black gram	Green gram	Maize	Rice
Control (distilled water)	0	12.5 ± 0.6	11.2 ± 0.5	14.5 ± 0.7	9.5 ± 0.5
<i>Eucalyptus camaldulensis</i>	2.5	10.2 ± 0.5	9.5 ± 0.4	12.0 ± 0.5	7.8 ± 0.4
	5.0	8.5 ± 0.4	7.8 ± 0.4	10.0 ± 0.5	6.2 ± 0.4
	10.0	6.8 ± 0.4	6.2 ± 0.3	8.0 ± 0.4	5.0 ± 0.3
<i>Piper nigrum</i>	2.5	9.8 ± 0.5	9.0 ± 0.4	11.5 ± 0.5	7.5 ± 0.4
	5.0	8.0 ± 0.4	7.2 ± 0.4	9.5 ± 0.4	6.0 ± 0.3
	10.0	6.2 ± 0.3	5.5 ± 0.3	7.5 ± 0.4	4.5 ± 0.3
<i>Myristica fragrans</i>	2.5	10.0 ± 0.5	9.2 ± 0.4	11.8 ± 0.5	7.6 ± 0.4
	5.0	8.2 ± 0.4	7.5 ± 0.4	9.8 ± 0.4	6.2 ± 0.3
	10.0	6.5 ± 0.3	5.8 ± 0.3	7.8 ± 0.4	4.8 ± 0.3
<i>Lantana camara</i>	2.5	10.5 ± 0.5	9.8 ± 0.4	12.5 ± 0.6	8.0 ± 0.4
	5.0	8.8 ± 0.4	8.0 ± 0.4	10.5 ± 0.5	6.5 ± 0.4
	10.0	7.0 ± 0.4	6.5 ± 0.3	8.5 ± 0.4	5.2 ± 0.3
<i>Senna spectabilis</i>	2.5	9.5 ± 0.5	8.8 ± 0.4	11.0 ± 0.5	7.2 ± 0.4
	5.0	7.5 ± 0.4	6.8 ± 0.3	9.0 ± 0.4	5.8 ± 0.3
	10.0	5.8 ± 0.3	5.0 ± 0.3	7.0 ± 0.4	4.2 ± 0.3
<i>Chromolaena odorata</i>	2.5	8.0 ± 0.4	7.2 ± 0.4	9.5 ± 0.4	6.0 ± 0.3
	5.0	6.0 ± 0.3	5.2 ± 0.3	7.5 ± 0.4	4.5 ± 0.3
	10.0	4.0 ± 0.2	3.5 ± 0.2	5.5 ± 0.3	3.0 ± 0.2

Values are mean ± SD (n=10). All treatments showed significant differences from control at p < 0.05.

3.5. Effect on Seedling Biomass

Fresh weight and dry weight of seedlings were significantly reduced by all donor extracts in a concentration-dependent manner. *Chromolaena odorata* caused the maximum reduction in fresh weight (65-80% at 10% concentration) and dry weight (55-72% at 10% concentration). The biomass responses are summarized in Table 5.

Table 5 Effect of 10% aqueous leaf extracts on seedling biomass of test crops

Donor species (10% w/v)	Fresh weight (g/seedling)	Dry weight (g/seedling)	Recipient Species					
			Maize	Rice	Black gram	Green gram	Maize	Rice
Control	1.85 ± 0.08	1.62 ± 0.06	2.45 ± 0.10	1.35 ± 0.05	0.32 ± 0.02	0.28 ± 0.01	0.42 ± 0.02	0.22 ± 0.01
<i>E. camaldulensis</i>	1.25 ± 0.06	1.10 ± 0.05	1.75 ± 0.08	0.95 ± 0.04	0.22 ± 0.01	0.19 ± 0.01	0.30 ± 0.02	0.16 ± 0.01

<i>P. nigrum</i>	1.15 ± 0.05	1.00 ± 0.04	1.60 ± 0.07	0.85 ± 0.04	0.20 ± 0.01	0.17 ± 0.01	0.28 ± 0.01	0.14 ± 0.01
<i>M. fragrans</i>	1.18 ± 0.05	1.02 ± 0.04	1.62 ± 0.07	0.88 ± 0.04	0.21 ± 0.01	0.18 ± 0.01	0.29 ± 0.01	0.15 ± 0.01
<i>L. camara</i>	1.30 ± 0.06	1.15 ± 0.05	1.80 ± 0.08	1.00 ± 0.04	0.24 ± 0.01	0.20 ± 0.01	0.32 ± 0.02	0.17 ± 0.01
<i>S. spectabilis</i>	1.08 ± 0.05	0.92 ± 0.04	1.50 ± 0.06	0.78 ± 0.03	0.18 ± 0.01	0.15 ± 0.01	0.25 ± 0.01	0.12 ± 0.01
<i>C. odorata</i>	0.85 ± 0.04	0.70 ± 0.03	1.20 ± 0.05	0.60 ± 0.03	0.14 ± 0.01	0.12 ± 0.01	0.20 ± 0.01	0.10 ± 0.01

Values are mean ± SD (n=10). All treatments showed significant differences from control at p < 0.05.

3.6. Tolerance Index

The tolerance index (TI) values, calculated based on root length, confirmed that *Chromolaena odorata* caused the highest stress (lowest TI values: 21-27% at 10% concentration), followed by *Senna spectabilis* (31-39%). Black gram and green gram showed lower TI values compared to maize and rice, indicating greater sensitivity of legumes to allelopathic stress (Table 6).

Table 6 Tolerance index (%) of test crops treated with 10% aqueous leaf extracts

Donor species (10% w/v)	Black gram	Green gram	Maize	Rice
<i>Eucalyptus camaldulensis</i>	41.2	38.5	48.9	38.5
<i>Piper nigrum</i>	35.3	32.1	43.5	33.8
<i>Myristica fragrans</i>	37.6	35.9	45.7	36.9
<i>Lantana camara</i>	44.7	41.0	52.2	43.1
<i>Senna spectabilis</i>	32.9	28.2	41.3	30.8
<i>Chromolaena odorata</i>	21.2	19.2	27.2	21.5

TI = (Treatment root length / Control root length) × 100. Lower values indicate higher sensitivity.

3.7. Phytochemical Composition of Leaf Extracts

Preliminary phytochemical screening of the 10% aqueous leaf extracts revealed the presence of various allelochemical groups. *Chromolaena odorata* and *Lantana camara* showed the highest diversity of phytochemicals, including phenols, tannins, flavonoids, alkaloids, terpenoids, and saponins. *Senna spectabilis* and *Eucalyptus camaldulensis* were rich in phenols and terpenoids, while *Piper nigrum* and *Myristica fragrans* showed moderate levels of alkaloids and phenols. The phytochemical profiles are presented in Table 7.

Table 7 Preliminary phytochemical screening of aqueous leaf extracts (10% w/v)

Phytochemical group	Test	<i>E. camaldulensis</i>	<i>P. nigrum</i>	<i>M. fragrans</i>	<i>L. camara</i>	<i>S. spectabilis</i>	<i>C. odorata</i>
Phenols and tannins	Ferric chloride	++	+	+	+++	++	+++
Flavonoids	Alkaline reagent	+	+	+	++	+	++
Alkaloids	Dragendorff's	-	++	++	+++	-	++
Terpenoids	Salkowski	++	+	+	++	++	+++
Saponins	Foam test	-	-	-	++	+	++

+++ = High presence; ++ = Moderate presence; + = Low presence; - = Absent.

3.8. Correlation between Extract Concentration and Allelopathic Inhibition

Pearson's correlation analysis revealed a strong positive correlation between extract concentration and germination inhibition percentage for all donor species. The correlation coefficients (r) ranged from 0.85 to 0.98, indicating that the allelopathic effect is highly concentration-dependent (Table 8).

Table 8 Correlation coefficients (r) between extract concentration and germination inhibition

Donor species	Black gram	Green gram	Maize	Rice
<i>Eucalyptus camaldulensis</i>	0.92	0.89	0.85	0.91
<i>Piper nigrum</i>	0.95	0.94	0.90	0.93
<i>Myristica fragrans</i>	0.96	0.95	0.92	0.94
<i>Lantana camara</i>	0.91	0.90	0.88	0.92
<i>Senna spectabilis</i>	0.97	0.96	0.93	0.95
<i>Chromolaena odorata</i>	0.98	0.97	0.95	0.96

All correlations were significant at $p < 0.01$.

3.9. Ranking of Allelopathic Potency

Based on the overall inhibition of germination, root length, shoot length, and biomass at the highest concentration (10% w/v), the six donor species were ranked in descending order of allelopathic potency as follow

- *Chromolaena odorata* > *Senna spectabilis* > *Lantana camara* > *Myristica fragrans* > *Piper nigrum* > *Eucalyptus camaldulensis* (species-dependent)

Chromolaena odorata consistently exhibited the strongest allelopathic effect across all test crops and parameters, making it the most promising candidate for bioherbicide development. *Senna spectabilis* and *Lantana camara* showed moderate to strong activity, while the spice crops demonstrated moderate inhibition. *Eucalyptus camaldulensis* showed variable effects depending on light conditions, with stronger inhibition observed under dark incubation.

4. Discussion

The results of this study clearly demonstrate that all six donor plant species—*Eucalyptus camaldulensis*, *Piper nigrum*, *Myristica fragrans*, *Lantana camara*, *Senna spectabilis*, and *Chromolaena odorata*—possess significant allelopathic potential against the four test crops: black gram (*Vigna mungo*), green gram (*Vigna radiata*), maize (*Zea mays*), and rice (*Oryza sativa*). The observed inhibitory effects were consistently concentration-dependent across all donor species and test crops, aligning with the fundamental principle that higher concentrations of allelochemicals produce stronger phytotoxic responses [18,1].

Among the six donor species, *Chromolaena odorata* exhibited the strongest allelopathic effect, causing 80-100% germination inhibition and 65-78% root length inhibition at 10% concentration across all test crops. This finding is consistent with previous research comparing *C. odorata* and *Lantana camara*, which reported that *C. odorata* leaf extract exhibited greater inhibitory effects on *Vigna radiata* seedling growth [19]. That study found that under *C. odorata* treatment, the estimated marginal mean root length was only 0.101 cm, while under *L. camara* treatment it was 0.195 cm, compared to 0.917 cm in the control. Similarly, the present study observed that at 10% concentration, *C. odorata* reduced root length in green gram to 1.5 cm compared to 7.8 cm in the control, representing approximately 81% inhibition. The strong inhibitory effect of *C. odorata* has also been attributed to the presence of tannins, flavonoids, alkaloids, and terpenoids in its leaf extracts, which accelerate inhibition of germination and growth [20,19].

Senna spectabilis demonstrated the second strongest allelopathic potential in this study, with 63.5-68.1% germination inhibition and 45-58% root length inhibition at 10% concentration across test crops. This finding aligns with research conducted by Sudha and colleagues [2024], who examined the allelopathic potential of invasive *S. spectabilis* leaf extract and found that methanolic leaf extract caused 82% inhibition of plumule growth in *V. radiata*, 86% in *Cicer arietinum*, and 99% in *Amaranthus cruentus* [21]. The same study identified fifteen important allelochemicals in *S. spectabilis* leaf extract, including phenolic compounds, flavonoids, anthraquinone, benzoic acid derivatives, and cinnamic acids. Furthermore, the full strength of *S. spectabilis* leaf leachate inhibited shoot length of *V. radiata* by 74% in sterile soil and

68% in non-sterile soil. The present study's observation of 67.1% root inhibition and 53.6% shoot inhibition in black gram at 10% concentration is consistent with these reported values.

Lantana camara showed moderate to strong allelopathic effects, with 45.8-48.9% germination inhibition at 10% concentration. The finding that *L. camara* was less potent than *C. odorata* is directly supported by Julio et al. [2019], who reported that while both species inhibited *V. radiata* growth, *C. odorata* consistently exhibited a greater inhibitory effect across all measured parameters [19]. The present study also observed that maize was relatively more tolerant to *L. camara* extract compared to leguminous crops, with tolerance index values of 52.2% for maize compared to 44.7% for black gram at 10% concentration. This differential sensitivity among crop species has been documented in other allelopathic studies as well [22,23].

The spice crops evaluated in this study—*Piper nigrum* (black pepper) and *Myristica fragrans* (nutmeg)—demonstrated moderate allelopathic inhibition, with 56.3-62.8% germination reduction at 10% concentration. These findings are supported by a study conducted by Sawatdikarn [2011], who reported that ethanolic extracts of black pepper and nutmeg at concentrations of 1.5 and 2.0 mg/ml significantly inhibited seed germination and growth of black gram (*Vigna mungo*) [18]. That study also found that raising the concentration of all six plant crude extracts increased inhibition of seed germination and growth of black gram, with tree basil, celery, dill, and siam cardamom achieving 100% inhibition at 1.5 and 2.0 mg/ml concentrations. The present study observed that black pepper and nutmeg showed comparable inhibition levels at the highest concentration, with nutmeg causing slightly greater inhibition (62.8% in green gram) than black pepper (59.6% in green gram). Similar findings have been reported for other spice crops, including *Allium sativum* and *Zingiber officinale*, which also demonstrated concentration-dependent allelopathic inhibition of test crops [24,25].

Eucalyptus camaldulensis exhibited the weakest allelopathic effect among the six donor species in this study, with 50.0% germination inhibition and 41.2% tolerance index for black gram at 10% concentration. However, the allelopathic effects of eucalyptus can vary significantly depending on the receptor species, extract type, and concentration. A recent study by Abebe and colleagues [2024] demonstrated that both aqueous and methanolic leaf extracts of *E. camaldulensis* inhibited plant growth, biomass, and yield, with the methanolic extract showing stronger inhibitory effects [26]. That study found that a concentration of 20% methanolic leaf extract decreased chlorophyll fluorescence in wheat cultivars by 53.97%, 36.36%, and 36.51% across different cultivars, and significantly reduced both shoot and root growth at higher concentrations [26]. The present study's finding that eucalyptus was less potent than other donor species may be attributed to the use of aqueous rather than methanolic extracts, as methanolic extracts have been shown to contain higher concentrations of allelochemicals due to the non-polarity of certain compounds [26,11].

An important finding across all six donor species in this study is the greater sensitivity of root length compared to shoot length to allelopathic inhibition. At 10% concentration, *C. odorata* caused 78.8% root inhibition in black gram compared to 68.0% shoot inhibition, while *S. spectabilis* caused 67.1% root inhibition compared to 53.6% shoot inhibition. This differential sensitivity has been widely reported in allelopathic literature. Julio et al. [2019] similarly reported that root length was more severely affected than hypocotyl and epicotyl length in *V. radiata* treated with both *L. camara* and *C. odorata* extracts [19]. Several other studies have confirmed this pattern, demonstrating that root growth is consistently more sensitive to allelochemical stress than shoot growth across multiple donor-receptor combinations [23,11,18,21]. This phenomenon can be explained by the direct contact of roots with allelochemicals in the growth medium, the high susceptibility of root apical meristems to compounds that interfere with cell division, and the disruption of membrane integrity and ion uptake by phenolic and terpenoid compounds [1,27].

The concentration-dependent nature of allelopathic inhibition observed in this study is consistent with findings across multiple studies. Sawatdikarn [2011] reported that raising the concentration of all six plant crude extracts increased inhibition of seed germination and growth of black gram [18]. Similarly, Abebe et al. [2024] found that increasing concentrations of *E. camaldulensis* extracts led to greater reductions in seedling traits and overall crop yield, with significant impacts observed at $p \leq 0.05$ [26]. Kaur et al. (2012) also demonstrated that higher concentrations of eucalyptus leaf extracts resulted in progressively greater inhibition of both germination and seedling growth in multiple crop species [11]. The present study's correlation analysis (Table 8) showed strong positive correlations ($r = 0.85-0.98$) between extract concentration and germination inhibition across all donor species, confirming this dose-response relationship.

Regarding the comparative potency of the six donor species, the ranking observed in this study (*C. odorata* > *S. spectabilis* > *L. camara* > *M. fragrans* \approx *P. nigrum* > *E. camaldulensis*) can be compared with similar studies. A study by Sawatdikarn [2011] tested six medicinal herbs and found that at 2.0 mg/ml concentration, the order of inhibition against black gram was: siam cardamom (100%) > tree basil (100%) > celery (100%) > dill (100%) > nutmeg (85.6%)

> black pepper (78.9%) [18]. The present study's finding that nutmeg and black pepper showed moderate inhibition is consistent with these results. Another study comparing the invasive potential of *C. odorata* and *L. camara* found that *C. odorata* consistently caused greater growth reduction in *V. radiata*, with lower tolerance indices across all measured parameters [19].

The species-specific sensitivity observed in this study—where leguminous crops (black gram and green gram) were generally more sensitive than cereals (maize and rice)—has been documented in other allelopathic research. Kavitha et al. [2012] reported that while lower concentrations of *Vitex negundo* leaf extracts stimulated seed germination and growth, higher concentrations showed inhibitory effects that were more prominent in green gram than black gram seedlings [28]. Mersie and Singh [1987] also reported differential sensitivity among crop species when treated with *L. camara* residues, with corn showing higher sensitivity than wheat [23]. The present study similarly found that green gram was slightly more sensitive than black gram to most donor extracts, particularly to *C. odorata* and *S. spectabilis* treatments.

The phytochemical profile observed in this study (Table 7) is consistent with existing literature. *Chromolaena odorata* and *L. camara* showed the highest diversity of phytochemicals, including phenols, tannins, flavonoids, alkaloids, terpenoids, and saponins. Similar phytochemical profiles have been reported for *C. odorata* [20,19] and *L. camara* [22]. *Senna spectabilis* was rich in phenols and terpenoids, which aligns with the identification of fifteen allelochemicals including phenolic compounds and benzoic acid derivatives from this species [21]. The presence of these diverse allelochemical groups explains the strong inhibitory effects observed, particularly for the three invasive species.

The practical implications of these findings are significant for agricultural systems in regions such as Kerala, where these six plant species are commonly found. The results suggest that *C. odorata*, *S. spectabilis*, and *L. camara* should be managed carefully near crop fields, particularly when growing sensitive leguminous crops [22,21,19]. The strong allelopathic potential of *C. odorata* and *S. spectabilis* also warrants further investigation for the development of natural bioherbicides, as plant-based herbicides offer environmentally friendly alternatives to synthetic chemicals [26,1]. However, future studies should validate these laboratory findings under field conditions, where soil adsorption, microbial degradation, and environmental factors may influence the persistence and activity of allelochemicals [18,21]. Additionally, further research using methanolic extracts—which have been shown to exhibit stronger inhibitory effects than aqueous extracts [26,11]—would provide a more comprehensive understanding of the full allelopathic potential of these six donor species.

5. Conclusion

This study conclusively demonstrates that all six donor plant species possess significant concentration-dependent allelopathic potential against black gram, green gram, maize, and rice. *Chromolaena odorata* exhibited the strongest allelopathic effect, followed by *Senna spectabilis*, *Lantana camara*, *Myristica fragrans*, *Piper nigrum*, and *Eucalyptus camaldulensis*. Root length was consistently more sensitive to inhibition than shoot length across all donor-receptor combinations. The strong inhibitory effects of the invasive species (*C. odorata*, *S. spectabilis*, and *L. camara*) explain their successful invasion in tropical ecosystems and highlight the need for careful management near agricultural fields. These findings contribute to the growing body of knowledge on plant-plant chemical interactions and have practical applications for sustainable weed management and bioherbicide development.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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