



Allelopathic potential of *Alternanthera philoxeroides* (Mart.) Griseb. on germination of black gram (*Vigna mungo* L.) and weed seeds

Ashwini Agarwalla^{1*}, S Kamala Bai^{2*}, K J Sowmya³, Ajay Abhishek⁴, Anjali Kailas Dhire⁵ & Thimmaiah Hosoklu⁶

¹Department of Agronomy, Assam Agricultural University, Jorhat 785 013, Assam, India

²All India Co-ordinated Research Project on Weed Management, University of Agricultural Sciences, Bengaluru 560 065, Karnataka, India

³Department of Seed Technology, University of Agricultural Sciences, Bengaluru 560 065, Karnataka, India

⁴Department of Soil Science and Water Management, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan 173 230, Himachal Pradesh, India

⁵Department of Agronomy, Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722, Maharashtra, India

⁶Department of Agronomy, University of Agricultural Sciences, Bengaluru 560 065, Karnataka, India

*Correspondence: Ashwini Agarwalla, ashwiniagarwalla789@gmail.com; S Kamala Bai, skamalabai@gmail.com

Abstract

The present study was conducted at the University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra (GKVK), Bengaluru in the year 2024 to evaluate the allelopathic efficacy of aqueous extracts of *Alternanthera philoxeroides* (Mart.) Griseb., at varying concentrations (120 and 160 g L⁻¹) on black gram and selected weed species. Optimised *in vitro* germination bioassays were conducted to assess their effects on seed germination, seedling growth and vigour. The results revealed a significant reduction in germination percentage, seedling length and seedling vigour index (SVI) of black gram and weed seeds following application of the aqueous extracts. The strongest inhibitory effect on germination of black gram and *Borreria hispida* (L.) K.Schum. was recorded with leaf extract at 160 g L⁻¹, whereas germination of *Echinochloa colona* (L.) Link was most severely affected by whole-plant extract at 120 g L⁻¹. Furthermore, root and shoot growth of *B. hispida* showed greater sensitivity to the extracts (0.09 and 0.16 cm, respectively) compared to *E. colona* (2.21 and 0.75 cm, respectively). The order of extract effectiveness in reducing SVI was leaf (0) > whole plant (0) > stem (2.01) > root (47.85) for *B. hispida* and whole plant (47.01) > leaf (60.99) > stem (64.80) > root (167) for *E. colona*. Gas chromatography-mass spectrometry (GC-MS) analysis of the extracts identified numerous allelopathic compounds with whole plant extract containing more phytoconstituents than other plant parts. These findings demonstrate the allelopathic potential of different plant-part extracts of *A. philoxeroides* and highlight their potential application as a bio-based tool for sustainable weed management.

Keywords: allelopathy; black gram; *Borreria hispida*; *Echinochloa colona*; weed control efficiency

Received: 24 March 2026. **Accepted:** 08 September 2026. **Available online:** Version 1.0: 22 October 2026.

Cite this article: Ashwini A, Kamala BS, Ajay A, Anjali KD, Thimmaiah H. Allelopathic potential of *Alternanthera philoxeroides* (Mart.) Griseb. on germination of black gram (*Vigna mungo* L.) and weed seeds. Plant Science Today. 2026;XX(X):1-XX. <https://doi.org/10.14719/pst.14680>

1. Introduction

Black gram (*Vigna mungo* L.), also known as urad bean, is a prominent pulse crop grown extensively in India. The crop occupies about 4.14 million ha in the country producing 2.23 million tonnes having productivity of 538 kg ha⁻¹. In Karnataka, black gram is cultivated in an area of 0.89 lakh hectares yielding 0.51 lakh tonnes with an average productivity of 576 kg per hectare (1). It is cultivated mainly for its seeds and forms an integral part of the vegetarian diet in the Indian subcontinent. It has high biological value and is an important source of easily digestible high-quality protein. However, it is less competitive against many weeds during the early stage of the crop and the most sensitive period with respect to

weed competition is between 15 to 45 days after sowing (2). Uncontrolled weeds can lead to significant decrease in black gram yield, with reduction of up to 41.20 % in the summer and 41.60 % during the kharif season (3). The common infesting weeds are *Echinochloa colona* (L.) Link, *Eleusine indica* (L.) Gaertn., *Digitaria marginata* Link, *Cynodon dactylon* (L.) Pers. among grasses, *Borreria hispida* (L.) K.Schum., *Commelina benghalensis* L., *Argemone mexicana* L. and *Portulaca oleracea* L. among broad-leaved weeds and one sedge, *Cyperus rotundus* L.

Borreria hispida belongs to the family Rubiaceae. This family comprises about 280 species distributed in tropical and subtropical America, Africa, Asia and Europe. It is widely distributed throughout India,

up to 900 m in hills and on all dry lands and is considered a problematic weed in agricultural fields. The plant is characterised by its prostrate to erect growth habit, small white flowers and hairy stems and leaves (4).

Echinochloa species are usually reported as noxious weeds in several economically important crops around the world. The most widespread species of the genus are *Echinochloa crus-galli* (L.) P. Beauv. and *E. colona*. *Echinochloa colona* is an erect, annual summer rhizomatous grass/weed, globally considered as one of the most troublesome weeds. It is native to tropical and subtropical Asia, Africa and Australia and competes with crops (5).

Weeds are often considered ubiquitous pests that interfere with the growth of cultivated crops through competition, allelomeditation and allelopathy (6). They cause substantial reductions in crop yields and overall productivity of field crops by competing for nutrients, moisture, light and space. Herbicides account for approximately two-thirds of global pesticide usage, underscoring the heavy reliance on them for combating weed infestations in agriculture. However, the widespread use of synthetic herbicides has led to several adverse effects, including their persistence in the environment, the rise of herbicide-resistant weeds and potential contamination of the food chain, which pose risks to human health. Allelochemicals obtained from plants may offer an environmentally sustainable approach for reducing the use of synthetic chemical inputs. Allelopathy is widely defined as an ecological phenomenon where plants gain a competitive edge through biochemical pathways. This occurs when secondary metabolites produced by plants, microorganisms, viruses and fungi affect the growth and development of other plants and organisms, either stimulating or inhibiting them (7–9). *Alternanthera philoxeroides*, also known as alligator weed, is a promising species in the search for new molecules with biological effects. It is a perennial, amphibious invasive plant native to South America (10). At least 60 different compounds have been isolated from the plant's aerial parts, with flavonoids and saponins being the primary classes. Other metabolites contributing to allelopathic activity include alkaloids, anthraquinones and megastigmanes.

Research into the effectiveness of organic herbicides and the allelopathic effects of plant extracts on weed dynamics has been relatively limited. This is an area that offers significant potential for developing sustainable weed management practices. So, there is need to identify low-cost, sustainable weed management methods that maintain both weed control efficiency and ecological significance, particularly for small-scale farmers and organic growers. Therefore, the present study was carried out with the following objectives: 1. To assess the effect of *A. philoxeroides* extract on germination, seedling growth and seedling vigour index (SVI) of black gram and selected weed species; 2. To study the metabolite profile of *A. philoxeroides* extract through gas chromatography-mass spectrometry (GC-MS) analysis; 3. To determine the most phytotoxic plant

part and concentration for potential weed management application.

2. Materials and Methods

The study was conducted in the Seed Science Laboratory, University of Agricultural Sciences (UAS), Gandhi Krishi Vigyan Kendra (GKVK), Bengaluru, Karnataka, India from June 2024 to November 2024. To conduct the experiment, alligator weed *A. philoxeroides* was collected from Puttenahalli Lake which is in Bengaluru. The fresh plant was thoroughly washed four times with running tap water and a final rinse with sterile water. The plant parts (leaves, stem, root and whole plant) were dissected into small pieces and dried under shade for 7 days to ensure they were dried completely. The dried plant materials were powdered in a plant sample grinder, sieved and stored in dark bags. For preparation of aqueous extract, 10 g of powdered plant material was mixed in 50 mL distilled water. The turbid solution was agitated on a mechanical shaker at 150 rpm for 24 hr. Following agitation, the solution was filtered through Whatman No. 1 filter paper and the pH was recorded, which ranged between 5 to 7. The filtered solution was then stored at 4 °C until use in the experiment. The experiment was laid out in completely randomised design (CRD) with 9 treatments as follows- T₁: Stem extract at 120 g L⁻¹, T₂: Stem extract at 160 g L⁻¹, T₃: Leaf extract at 120 g L⁻¹, T₄: Leaf extract at 160 g L⁻¹, T₅: Root Extract at 120 g L⁻¹, T₆: Root Extract at 160 g L⁻¹, T₇: Whole plant Extract at 120 g L⁻¹, T₈: Whole plant Extract at 160 g L⁻¹ and T₉: Control. Prior to the main experiment, a preliminary screening trial was conducted to standardise the effective phytotoxic concentration of the plant extracts. Laboratory bioassays were carried out with leaf, stem, root and whole plant extracts at twelve concentrations (2, 5, 10, 20, 40, 60, 80, 100, 120, 140, 160 and 180 g L⁻¹) using germination percentage and SVI of black gram as response criteria. No significant inhibition of germination was observed at concentrations below 100 g L⁻¹ in comparison to control, while concentrations above 160 g L⁻¹ resulted in an almost complete inhibition of germination and prevented recording meaningful growth measurements. Based on this dose-response trend, the concentrations of 120 and 160 g L⁻¹ were identified as those producing significant, measurable and reproducible phytotoxic effects and were therefore chosen for detailed evaluation in the main experiment.

To conduct the experiment, mature seeds of major weed species namely *B. hispidula*, *E. indica*, *D. marginata* and *E. colona* were collected from the experimental fields of UAS, GKVK, Bengaluru during May, 2024. Healthy seeds were imbibed at room temperature (25 °C) for 5 hr and then subjected to different dormancy treatments. Treatments to break dormancy included: 1. Seeds were immersed in hot water at 90 °C for 10 min. Following hot water immersion, the seeds were rapidly cooled by placing them under running cold water. 2. Chemical scarification: a) Seed samples were placed in concentrated sulphuric acid for 2 min. Following acid treatment, seeds were washed in running



water until the pH was neutral and then rinsed in sterile water; thereafter, the seeds were immersed in 0.2 % KNO_3 solution for 12 hr. 3. Pre-chilling at 4 °C for one week. 4. Control. About 10–15 seeds were placed in 10 cm Petri dishes lined with two sheets of Whatman No. 1 filter paper and containing 7 mL distilled water. There were 3 replicates for each treatment. The seeds were left in an incubator to germinate at a constant temperature of 25 °C. The number of seeds germinated were counted 7 days after treatment imposition (11). The plates showing the highest germination were considered the best dormancy-breaking treatment and were subsequently subjected to experimental treatments. In the study, 10–15 pretreated seeds with 0.2 % potassium nitrate (KNO_3) solution, were placed in glass Petri dishes lined with sterilised filter paper. Each Petri dish was then filled with 5 mL of the extract as specified by the treatment protocol. Radicle emergence was considered an index of germination and observed at 24 hr intervals for 7 days and expressed as per cent seed germination. Distilled water was used as control.

Germination percentage

Fifty seeds of black gram (LBG-791) in 4 replicates were placed on germination paper and 10 mL of extract was added to each germination paper as per treatment and the germination test was conducted using between paper methods as per (12). The rolled towels were incubated in germination chamber maintained at 25 ± 1 °C and 90 % relative humidity. Distilled water treatment served as control. The final count of the germinated seedlings was taken on 7th day and germination percent was expressed based on normal seedlings.

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Seedling length (cm)

Ten normal seedlings were taken randomly from the treatments and replications from paper towel of laboratory germination test and total length of seedling was measured using metric scale. The mean of the ten seedlings in each treatment and replication was calculated and expressed in centimetres. Three seedlings from each Petri dish were selected randomly and the length of radicle and plumule was measured with the help of a scale for the both the weed species.

Seedling vigour index

Seedling vigour index is a measure used to evaluate the overall performance and quality of seedlings by combining seed germination percentage with seedling growth. It indicates the potential of seeds to produce strong, healthy seedlings under favourable conditions. It is calculated as suggested by (13).

$$\text{SVI} = (\text{Shoot length} + \text{Root length}) \times \text{Germination percentage}$$

Gas chromatography-mass spectrometry metabolite profiling

Sample preparation

Aliquots (2 mL) of *A. philoxeroides* extracts were centrifuged (10000 rpm, 15 min), diluted 20-fold and extracted with ethyl acetate (1 : 1 v/v). The resulting extract was concentrated to 1 mL using a rotary evaporator at 55 °C and immediately subjected to GC-MS analysis.

Instrumentation and analysis

Analysis was performed using an Elite-5MS column (30 m × 250 μm; 5 % phenyl 95 % dimethylpolysiloxane). Helium (99.99 %) served as the carrier gas at a constant flow rate of 1 mL min⁻¹. The oven temperature was programmed from 50 °C (initial) to 220 °C at 8 °C min⁻¹ (held for 5 min), then increased to 280 °C at 7 °C min⁻¹ (held for 15 min). A 2 μL sample was injected at 280 °C with a split ratio of 10 : 1.

Mass spectrometry and identification

The MS was operated in electron ionisation mode (70 eV) with a scan range of 40–600 amu. The inlet and source temperatures were maintained at 200 °C and 150 °C, respectively. Total run time was 38 min. Compounds were identified by comparing mass spectra with the NIST 2005 library.

Statistical analyses

The experimental data obtained on various seed quality parameters were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test prior to analysis. The data were then subjected to Fisher's method of "Analysis of Variance" as outlined by previous researchers (14). The standard errors of mean were calculated for each parameter and critical differences (CD) at 1 % were worked out using the least significant test for comparing the treatment means wherever 'F' test was found significant.

3. Results

Effect of aqueous extracts on black gram seeds

The data on germination percentage and SVI of black gram seeds are presented in Fig 1. Significant differences were noted among the treatments over control. Leaf extract at 160 g L⁻¹ recorded significantly lower germination percentage (64.33 %) followed by stem extract at 120 g L⁻¹ (65.33 %) whereas the control recorded highest germination percentage (84.00 %). Root length decreased with increase in concentration of different plant parts except for the root extract; however, it was significant over control (Table 1). Significantly lower root length (12.91 cm) was recorded with stem extract at 160 g L⁻¹ followed by root extract at 120 g L⁻¹ (13.10 cm). Increase in concentration led to a significant increase in shoot length except for whole plant extract. Maximum shoot length (17.63 cm) was recorded with root extract at 160 g L⁻¹ and minimum with stem extract at 120 g L⁻¹ (9.75 cm). The highest inhibition in seedling length was recorded in stem extract at 160 g L⁻¹ (23.03 cm) (Table 1). The SVI decreased with increasing concentration,



except for root extract at 160 g L⁻¹ (2217). However, it was significant over control which recorded SVI of 3042.

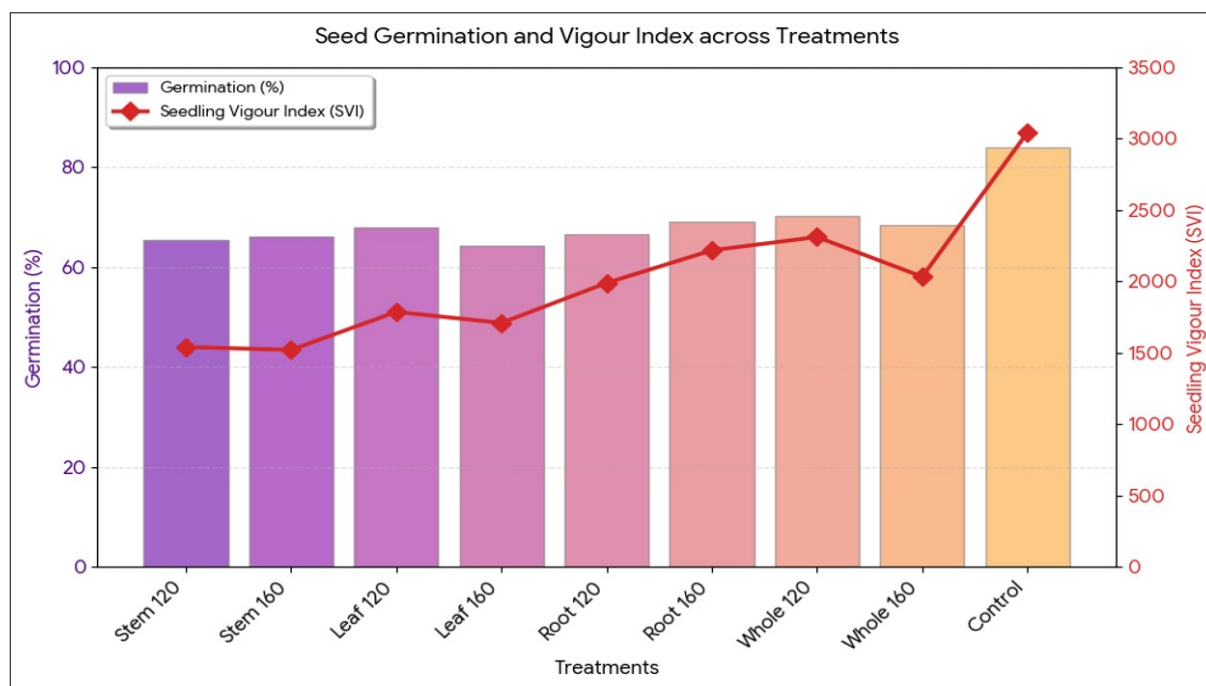


Fig. 1. Influence of different plant part extracts on seed germination (%) and seedling vigour index (SVI) of black gram (*Vigna mungo*).

Table 1. Effect of aqueous extracts of *Alternanthera philoxeroides* plant parts on shoot length, root length and seedling length of black gram

Treatments	Shoot length (cm)	Root length(cm)	Seedling length (cm)
T ₁ : Stem extract at 120 g L ⁻¹	9.75	13.82	23.57
T ₂ : Stem extract at 160 g L ⁻¹	10.12	12.91	23.03
T ₃ : Leaf extract at 120 g L ⁻¹	11.31	14.94	26.25
T ₄ : Leaf extract at 160 g L ⁻¹	12.70	13.86	26.56
T ₅ : Root extract at 120 g L ⁻¹	16.72	13.10	29.82
T ₆ : Root extract at 160 g L ⁻¹	17.63	14.51	32.14
T ₇ : Whole plant extract at 120 g L ⁻¹	14.64	18.20	32.84
T ₈ : Whole plant extract at 160 g L ⁻¹	12.93	16.82	29.75
T ₉ : Control	16.90	19.32	36.22
S. Em. ±	0.38	0.42	0.80
C.D. (p = 0.01)	1.14	1.27	2.40

Effect of aqueous extract on weed seeds

A germination test was conducted on the major weed seeds, whose density was higher as compared to other weed seeds per square meter in field experiment. Major weed seeds subjected to germination were *E. colona*, *E. indica*, *B. hispida* and *D. marginata*. Among the weed seeds, only *B. hispida* and *E. colona* germination was observed, while in other weed seeds there was complete failure of germination. Aqueous extracts of all plant parts of *A. philoxeroides* exhibited marked allelopathic activity, however, their phytotoxicity varied with plant part and concentration. Whole plant extract exerted the strongest inhibitory effect, followed by leaf extract, with higher concentrations (160 g L⁻¹) causing greater suppression of

germination (Table 2 and 3). Complete inhibition of germination in *B. hispida* was observed in leaf extract at 160 g L⁻¹ and whole plant extract at 120 g L⁻¹ compared to 51.00 % germination in the control. In *E. colona*, the lowest germination percent were recorded with whole plant extract at 120 g L⁻¹ (16.67 %) and 160 g L⁻¹ (26.67 %), as against 56.70 % in control. The aqueous extracts of *A. philoxeroides* exerted strong and differential allelopathic effects on the early growth of *B. hispida* and *E. colona*. In *B. hispida*, application of leaf extract at 160 g L⁻¹ and whole plant extract at 120 g L⁻¹ resulted in complete inhibition of shoot, root and seedling growth, indicating higher sensitivity of this species to allelochemicals. However, the SVI was most severely reduced by aqueous extracts in the order leaf > whole plant > stem > root, further confirming

the higher phytotoxic potential of leaf-derived allelochemicals (Fig. 2).

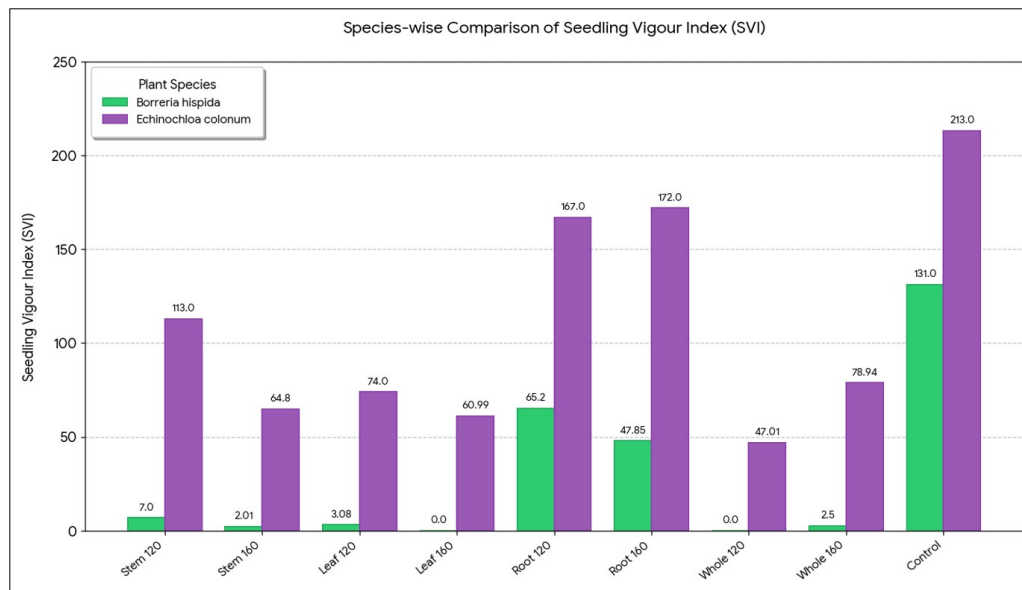


Fig. 2. Comparative seedling vigour index (SVI) of *Borreria hispida* and *Echinochloa colona* as influenced by different plant part extracts and concentrations.

Table 2. Effect of aqueous extracts of *Alternanthera philoxeroides* plant parts on per cent germination, root length, shoot length and seedling length of *Borreria hispida*

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)
T ₁ : Stem extract at 120 g L ⁻¹	18.42 (10.00)	0.28	0.42	0.70
T ₂ : Stem extract at 160 g L ⁻¹	10.51 (3.33)	0.20	0.40	0.60
T ₃ : Leaf extract at 120 g L ⁻¹	10.51 (3.33)	0.20	0.73	0.93
T ₄ : Leaf extract at 160 g L ⁻¹	0.00 (0.00)	0.00	0.00	0.00
T ₅ : Root extract at 120 g L ⁻¹	39.21 (40.00)	0.88	0.75	1.63
T ₆ : Root extract at 160 g L ⁻¹	35.00 (33.00)	0.76	0.69	1.45
T ₇ : Whole plant extract at 120 g L ⁻¹	0.00 (0.00)	0.00	0.00	0.00
T ₈ : Whole plant extract at 160 g L ⁻¹	18.41 (10.00)	0.09	0.16	0.25
T ₉ : Control	45.51 (51.00)	1.09	1.48	2.31
S. Em. (±)	0.28	0.02	0.02	0.06
C.D. (<i>p</i> = 0.01)	0.85	0.05	0.06	0.19

Data subjected to angular transformation. Figure in parenthesis shows the original values.

Table 3. Effect of aqueous extracts of *Alternanthera philoxeroides* plant parts on per cent germination, root length, shoot length and seedling length of *Echinochloa colona*

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)
T ₁ : Stem extract at 120 g L ⁻¹	39.21 (40.00)	1.84	0.99	2.83
T ₂ : Stem extract at 160 g L ⁻¹	33.23 (30.00)	1.39	0.77	2.16
T ₃ : Leaf extract at 120 g L ⁻¹	39.25 (40.00)	1.11	0.74	1.85
T ₄ : Leaf extract at 160 g L ⁻¹	35.22 (33.33)	1.16	0.67	1.83
T ₅ : Root extract at 120 g L ⁻¹	50.89 (60.00)	1.75	1.04	2.79
T ₆ : Root extract at 160 g L ⁻¹	46.90 (53.67)	2.35	0.85	3.20
T ₇ : Whole plant extract at 120 g L ⁻¹	24.32 (16.67)	1.91	0.91	2.82
T ₈ : Whole plant extract at 160 g L ⁻¹	31.13 (26.67)	2.21	0.75	2.96
T ₉ : Control	49.01 (56.67)	2.33	1.43	3.76
S. Em. (±)	0.77	0.07	0.03	0.07
C.D. (<i>p</i> = 0.01)	2.20	0.21	0.09	0.23

Data subjected to angular transformation. Figure in parenthesis shows the original values.

In contrast, *E. colona* exhibited a distinct species-specific response. Shoot growth was significantly inhibited with the leaf extract at 160 g L⁻¹ recording the lowest shoot length (0.67 cm) and the greatest reduction in seedling length (1.83 cm); however, it remained statistically at par with the whole plant extract at the same concentration, which recorded a shoot length of 0.75 cm (Table 3). These results suggest that shoot tissues in *E. colona* are sensitive to higher concentrations of allelochemicals, though to a lesser extent than *B. hispida*. Except under stem extract at 160 g L⁻¹, higher concentrations of extract from different plant parts of *A. philoxeroides* stimulated root elongation, indicating a differential allocation of growth responses between shoot and root systems. In *E. colona*, the order of effectiveness in reducing SVI followed whole plant > leaf > stem > root (Fig. 2), suggesting a cumulative or synergistic effect of allelochemicals when multiple plant tissues were involved.

Gas chromatography-mass spectrometry analysis

The GC-MS is one of the precise methods for identifying a wide range of secondary metabolites in plant extracts (15, 16). According to present findings, allelochemicals contained in the donor plant *A. philoxeroides* were

identified and quantified using GC-MS to evaluate the bioactivity of these substances. The compounds identified in the ethyl acetate (EtOAc) extracts in different parts of *A. philoxeroides* are presented in (Tables 4 and 5). The preliminary phytochemical analysis showed the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, amino acids, diterpenes and quinone in the extracts of *A. philoxeroides*. A total of 100 metabolites were detected in each plant part. Among these, only 10 compounds were selected as potential allelopathic phytoconstituents based on their reported allelopathic activity in the EtOAc extract of the stem, 10 in root, 15 in the leaf and 18 phytoconstituents with allelopathic effect in whole plant. The presence of these phytoconstituents justifies the use of this plant as natural bioherbicide. These results align with the findings of previous researchers who reported the presence of similar allelochemicals viz., alkaloids and phenols in ethyl acetate extract of *A. philoxeroides* (17). The presence of these phytoconstituents justifies the use of this plant as natural bioherbicide. These results align with the previous findings which reported the presence of similar allelochemicals viz., alkaloids and phenols in ethyl acetate extract of *A. philoxeroides* (18).

Table 4. Phytochemicals detected in ethyl acetate (EtOAc) extract in stem and root of *Alternanthera philoxeroides* by gas chromatography-mass spectrometry (GC-MS)

Stem				Root			
Retention Time	Peak area %	Similarity index (SI) %	Phytochemical	Retention Time	Peak area %	Similarity index (SI) %	Phytochemical
8.233	0.57	96	D-Limonene	8.23	1.13	96	D-Limonene
15.12	0.67	95	2,4-Di-tert-butylphenol	15.12	0.74	95	2,4-Di-tert-butylphenol
3.85	0.25	96	Toluene	15.42	0.14	90	Eicosane
19.78	0.30	92	Hexadecanoic acid, methyl ester	22.26	1.43	96	Hexadecanoic acid, butyl ester
20.15	2.29	90	n-Hexadecanoic acid	21.28	0.49	91	Cyclohexane, tetradecyl
21.57	6.49	94	Phytol	23.16	0.19	91	Cyclohexane, hexadecyl
22.54	1.18	95	Hexadecanoic acid, butyl ester	11.00	0.33	90	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl)
24.12	0.76	93	Octadecanoic acid, butyl ester	14.41	0.42	95	Cyclohexane, octyl
25.92	0.99	95	Bis(2-ethylhexyl) phthalate	24.12	0.72	92	Octadecanoic acid, butyl ester
11.00	0.23	90	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl)	24.21	0.61	95	Octacosanol

Table 5. Phytochemicals detected in ethyl acetate (EtOAc) extract in leaf and whole plant part of *Alternanthera philoxeroides* by gas chromatography-mass spectrometry (GC-MS)

Leaf				Whole plant			
Retention time	Peak area %	Similarity index (SI) %	Phytochemical	Retention time	Peak area %	Similarity index (SI) %	Phytochemical
8.22	3.66	97	D-Limonene	3.85	0.30	96	Toluene
7.56	0.21	95	1-Decene	8.23	3.10	96	D-Limonene



3.82	0.34	96	Toluene	15.12	0.64	95	2,4-Di-tert-butylphenol
13.71	1.02	97	Tetradecane	11.00	0.19	90	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanedily)
13.77	0.96	95	1-Tetradecene	11.53	0.33	95	Cyclohexane, hexyl
15.11	0.71	94	2,4-Di-tert-butylphenol	12.83	0.13	90	Cyclohexane, 1,2,4,5-tetraethyl
11.00	0.26	90	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethane)	14.41	0.43	96	Cyclohexane, octyl
16.95	0.57	93	Cyclohexane, undecyl	16.95	0.45	96	Cyclohexane, decyl
11.54	0.39	95	Cyclohexane, hexyl	21.28	0.46	93	Cyclohexane, tetradecyl
22.26	0.89	95	Hexadecanoic acid, butyl ester	19.78	0.26	90	Hexadecanoic acid, methyl ester
22.34	1.28	96	n-Tetracosanol	20.15	1.13	92	n-Hexadecanoic acid
24.12	0.58	92	Octadecanoic acid, butyl ester	22.33	1.74	96	1-Nonadecene
24.21	0.55	96	1-Heptacosanol	24.12	0.84	92	Octadecanoic acid, butyl ester
25.93	0.75	96	Bis(2-ethylhexyl) phthalate	22.25	1.60	95	Hexadecanoic acid, butyl ester
29.44	2.26	94	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	25.93	1.17	96	Bis(2-ethylhexyl) phthalate
				7.57	0.15	93	1-Decene
				13.72	1.07	98	Tetradecane
				29.43	3.35	95	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl)

4. Discussion

Germination represents a crucial initial stage in plant growth and development and is therefore a key indicator for assessing allelopathic activity. The aqueous extract of *A. philoxeroides* releases allelochemicals that interfere with essential biochemical processes during seed germination, thereby inhibiting germination and imposing environmental stress on the growth and development of black gram seedlings (18). Root length in black gram decreased with increasing extract concentration (160 g L⁻¹), except for root extract; however, it was significant over control. This reduction in root growth may be attributed to rapid inhibitory effect of allelochemicals on respiration of root tips because roots first come in contact with allelochemicals and are the first to absorb them from the environment in which they are growing. However, results for shoot length showed a contrasting pattern. Results agree with the earlier reports showing that lettuce shoot growth parameters increased while root growth declined in response to aqueous plant extracts, indicating that shoots are less sensitive to allelochemicals than roots (19). The reduction in seedling length and SVI could be attributed to water soluble allelochemicals mainly phenolic compounds that are released from *A. philoxeroides* and had phytotoxic impact on black gram seeds. In some plant part extracts viz., root and whole plant extract a lower dose of allelochemicals (120 g

L⁻¹) showed a stimulatory effect while higher concentration (160 g L⁻¹) was lethal. A similar dose-dependent response was reported by previous researchers wherein *Acacia nilotica* (L.) Willd. ex Delile leaf extract promoted pea growth at lower concentrations but exerted inhibitory effects at higher doses (20).

Application of aqueous extract at higher concentration significantly reduced the germination of both the weed species. The results of present study are in accordance with those of previous studies which stated when sensitive plants contact with allelopathic chemicals, it inhibits the germination of seeds and reduces their density (21). The preliminary changes that occur after the imbibition are due to a boost in the hydrolytic enzymes, such as α -amylase and protease. α -amylase is an imperative starch digestive enzyme in the seed. The blockage of seed germination is due to an interruption in the actions of peroxidase, α -amylase and acid phosphates. Aqueous extracts of all the plant parts of *A. philoxeroides* exhibited marked allelopathic activity against weed seeds; however, their phytotoxicity varied with plant part and concentration. In *B. hispida*, application of leaf extract at 160 g L⁻¹ and whole plant extract at 120 g L⁻¹ resulted in complete inhibition of shoot, root and seedling growth, indicating extreme sensitivity of this species to allelochemicals released from these plant parts. The inhibitory effect is likely attributable to the phytotoxic action of water-soluble allelochemicals, which



disrupt key physiological and metabolic processes involved in seed germination and seedling development (22). The greater efficacy of whole plant extract may be explained by the combined presence of allelochemicals from leaves, stems and roots, which might substitute one another due to their high biological exchange rate resulting in the potency increment to each other (23). The total suppression of growth parameters at higher concentration reflects severe disruption of fundamental physiological processes required for seedling establishment, such as cellular respiration, membrane permeability and enzymatic activity. But in *E. colona*, higher concentrations of extracts from most plant parts stimulated root elongation, except under stem extract at 160 g L⁻¹, indicating a differential allocation of growth responses between shoot and root systems. This stimulation of root growth may represent a hormetic response, wherein exposure to allelochemicals at certain concentrations enhances adaptive physiological processes. Enhanced root elongation can be attributed to increased rates of cell division, elevated protein synthesis, activation of enzymatic metabolism and improved water uptake during early seedling growth, as previously documented (24). The variation in extract effectiveness in reducing SVI further emphasises differences in allelochemical composition among plant parts and species-specific tolerance mechanisms. Overall, these findings demonstrate that while *A. philoxeroides* extracts possess strong allelopathic potential, their impact is highly species-dependent, with *B. hispida* showing pronounced susceptibility and *E. colona*, exhibiting partial tolerance through adaptive root responses. The differential allelopathic response exhibited by *B. hispida* and *E. colona* could be due to differences in seed coat permeability, antioxidant defence systems and enzymatic detoxification capacity between two weed species.

Putative allelopathic mechanisms of identified phytoconstituents

The GC-MS analysis identified several bioactive compounds with known allelopathic potential. D-Limonene, a monoterpene found in all plant parts of *A. philoxeroides*, can interact with the lipid bilayer, thereby disrupting the integrity of cell membrane, increasing permeability and causing leakage of cellular contents (25). Besides, it also affects mitochondrial respiration and ATP synthesis, leading to inhibition of seed germination and early seedling growth. 2,4-Di-tert-butylphenol is a phenolic compound identified in stem, root, leaf and whole plant extracts with a potent inhibitory effect on cell division since it disrupts microtubule assembly during mitosis and alters auxin transport pathways (26). Phenolic compounds are known allelochemicals that generate reactive oxygen species (ROS) and thus induce oxidative stress, resulting in lipid peroxidation and protein denaturation in target cells. Hexadecanoic acid (palmitic acid) and its esters were found in all plant part extracts and they affect the membrane fluidity and permeability by being incorporated in the phospholipid membranes, thus changing the ion transport and enzymatic activities associated with germination (27). Phytol, a diterpene alcohol in the stem

extract, is a chlorophyll derivative that has been shown to interfere with photosynthetic apparatus and electron transport chain in emerging seedlings, as well as inhibit gibberellin biosynthesis, which is essential for seed germination (28).

5. Conclusion

The findings of present study demonstrated the significant allelopathic potential of *Alternanthera philoxeroides* and highlight its potential as a sustainable bioherbicidal resource for eco-friendly weed management. The aqueous extracts exhibited concentration-dependent inhibitory effects on seed germination and seedling growth, with higher concentrations (160 g L⁻¹) yielding greater suppression. Among the plant parts evaluated, leaf and whole-plant extracts showed the highest allelopathic activity. In black gram, the lowest germination percentage was recorded with the leaf extract at 160 g L⁻¹ (64.33 %), followed by the stem extract at 120 g L⁻¹ (65.33 %). In *Borreria hispida*, complete inhibition of germination was achieved with the leaf extract at 160 g L⁻¹ and the whole-plant extract at 120 g L⁻¹, compared with 51.00 percentage germination in the control. Similarly, in *Echinochloa colona*, the whole-plant extract at 120 g L⁻¹ resulted in the lowest germination (16.67 %), followed by 160 g L⁻¹ (26.67 %), compared with 56.70 percentage in control. Root extract showed the lowest inhibitory activity at 120 g L⁻¹ and exhibited stimulatory effects on shoot elongation at lower concentrations indicating a hormetic response. *Borreria hispida* recorded significantly higher sensitivity (42–100 % growth inhibition) compared with *E. colona*, which showed a partial tolerance by adaptive root elongation. Gas chromatography-mass spectrometry (GC-MS) profiling showed the presence of 18 allelopathic phytoconstituents in whole plant extract including D-limonene, 2,4-di-tert-butylphenol, hexadecanoic acid and phytol which acts by disturbing membrane integrity, cell division, respiration and photosynthesis and inducing oxidative stress. The study provides valuable evidence supporting the utilisation of this invasive weed as a natural source of bioherbicidal compounds, thereby converting a problematic invasive species into a useful component of sustainable and environmentally friendly weed management strategies. The adoption of such plant-based bioherbicides could reduce dependence on synthetic herbicides, minimise environmental contamination and help mitigate the development of herbicide-resistant weed populations.

Future research should focus on the isolation, identification and characterisation of the specific allelochemicals responsible for the observed phytotoxic effects, molecular studies to elucidate the precise mode of action, development of stable nano-formulations or emulsions of the extracts to improve shelf life as well as application efficiency and evaluation of their selectivity towards crops and weeds. In addition, investigating combinations of *A. philoxeroides* with other allelopathic plant species may reveal synergistic interactions that enhance weed suppression, broaden the spectrum of



activity and reduce the quantity of plant material required. Such integrated approaches could facilitate the development of effective, economically viable and environmentally sustainable bioherbicidal formulations for future weed management programs.

Acknowledgements

The authors would like to express their sincere gratitude to the University of Agricultural Sciences, Gandhi Krishi Vigyan Kendra, Bengaluru, Karnataka, India, for providing the necessary resources and facilities to conduct this research.

Authors' contributions

AA¹ conceived the concept and conducted the research. SKB contributed to supervision of the research project. AA¹, SKB and AA² contributed to original draft writing, data analysis, software and visualisation. KJS participated in data recording for the bioassay studies. AKD and TH contributed to reviewing and editing. All authors have read and approved the final manuscript [AA¹ stands for Ashwini Agarwalla and AA² stands for Ajay Abhishek].

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

References

- Directorate of Economics and Statistics. Bengaluru: Government of Karnataka; 2020.
- Choudhary VK, Kumar SP, Bhagawati R. Integrated weed management in black gram (*Vigna mungo*) under mid hills of Arunachal Pradesh. *Indian J Agron*. 2012;57(4):382–5. <https://doi.org/10.59797/ija.v57i4.4651>
- Singh G. Weed management in summer and kharif season blackgram (*Vigna mungo* (L.) Hepper). *Indian J Weed Sci*. 2011;43(1-2):77–80.
- Muthu CSAK. Various extracts of whole plant of *Borreria hispida* (Linn). *Int J Pharm Bio Sci*. 2010;1(3):1–11.
- Peerzada AM, Bajwa AA, Ali HH, Chauhan BS. Biology, impact and management of *Echinochloa colona* (L.) Link. *Crop Prot*. 2016;83:56–66. <https://doi.org/10.1016/j.cropro.2016.01.011>
- Weston LA, Duke SO. Weed and crop allelopathy. *Crit Rev Plant Sci*. 2003;22(3-4):367–89. <https://doi.org/10.1080/713610861>
- Macias FA, Molinillo JG, Varela RM, Galindo JG. Allelopathy-a natural alternative for weed control. *Pest Manag Sci*. 2007;63(4):327–48. <https://doi.org/10.1002/ps.1342>
- Nakai S, Zou G, Okuda T, Nishijima W, Hosomi M, Okada M. Polyphenols and fatty acids responsible for anti-cyanobacterial allelopathic effects of submerged macrophyte *Myriophyllum spicatum*. *Water Sci Technol*. 2012;66(5):993–9. <https://doi.org/10.2166/wst.2012.272>
- Rimando AM, Olofsdotter M, Dayan FE, Duke SO. Searching for rice allelochemicals: an example of bioassay guided isolation. *Agron J*. 2001;93(1):16–20. <https://doi.org/10.2134/agronj2001.93116x>
- Maheshwari JK. Alligator weed in Indian lakes. *Nature*. 1965;206(4990):1270. <https://doi.org/10.1038/2061270a0>
- Sadeghi S, Ashrafi ZY, Tabatabai F, Alizadeh HM. Study methods of dormancy breaking and germination of common madder (*Rubia tinctorum* L.) seed in laboratory conditions. *Bot Res Int*. 2009;2(1):7–10.
- Anonymous. International rules for seed testing. Switzerland: The International Seed Testing Association (ISTA); 2013.
- Abdul-Baki AA, Anderson JD. Vigour determination in soybean seed by multiple criteria. *Crop Sci*. 1973;13(6):630–3. <https://doi.org/10.2135/cropsci1973.0011183X00130006013x>
- Gomez KA, Gomez AA. Statistical procedures for agricultural research. New York: Wiley and Sons; 1984. p. 680.
- Deshpande SN, Kadam DG. GCMS analysis and antibacterial activity of *Piper betle* (Linn) leaves against *Streptococcus mutans*. *Asian J Pharm Clin Res*. 2013;6(Suppl 5):99–101.
- Payum T. GC-MS analysis of *Mussaenda roxburghii* Hk. f.: a folk food plant used among tribes of Arunachal Pradesh, India. *Pharmacogn J*. 2016;8(4):395–8. <https://doi.org/10.5530/pj.2016.4.14>
- Kandhro MN, Tunio SD, Rajpar I, Chachar QD, Gandahi AW. Allelopathic impact of sorghum and sunflower on germination and seedling growth of summer broadleaf weeds. *Pak J Agric Sci*. 2015;31(2):229–39.
- Kamala Bai SK, Avinash G, Sindhu KK, Geetha KN. Allelopathic potential of *Alternanthera philoxeroides* (Mart.) Griseb on growth and development of *Eichhornia crassipes* (Mart) Solms. *Allelopathy J*. 2023;60(2):159–70. <https://doi.org/10.26651/allelo.j/2023-60-2-1461>
- Rezaeinodehi A, Khangholi S, Aminidehagh M, Kazemi H. Allelopathic potential of tea (*Camellia sinensis* (L.) Kuntze) on germination and growth of *Amaranthus retroflexus* L. and *Setaria glauca* (L.) P. Beauv. *J Plant Dis Prot*. 2006;20:447–54.
- Wang K, Wang T, Ren C, Dou P, Miao Z, Liu X, et al. Aqueous extracts of three herbs allelopathically inhibit lettuce germination but promote seedling growth at low concentrations. *Plants*. 2022;11(4):486. <https://doi.org/10.3390/plants11040486>
- Al-Wakeel SAM, Gabr MA, Hamid AA, Abu-El-Soud WM. Allelopathic of *Acacia nilotica* leaf residue on *Pisum sativum* L. *Allelopathy J*. 2007;19(2):411–22.
- Arif M, Cheema ZA, Khaliq A, Hassan A. Organic weed management in wheat through allelopathy. *Int J Agric Biol*. 2015;17(1):127–34.



23. Gerig TM, Blum U. Effects of mixtures of four phenolic acids on leaf area expansion of cucumber seedlings grown in Portsmouth BI soil materials. *J Chem Ecol.* 1991;17(1):29–39. <https://doi.org/10.1007/BF00994420>
24. Ramachandran A. Efficacy of different botanical extracts on the management of *Parthenium hysterophorus* (L.). *J Res Weed Sci.* 2019;2(1):16–32.
25. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a review. *Food Chem Toxicol.* 2008;46(2):446–75. <https://doi.org/10.1016/j.fct.2007.09.106>
26. Zhao F, Cheng Y, Zhang Y, Qiu L, Zhang X, Zhao J, et al. Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. *Toxins.* 2020;12(1):35. <https://doi.org/10.3390/toxins12010035>
27. Hac-Wydro K, Wydro P, Jagoda A. The influence of fatty acids on model cholesterol/phospholipid membranes. *Chem Phys Lipids.* 2007;150(2):66–81. <https://doi.org/10.1016/j.chemphyslip.2007.06.213>
28. McGarvey DJ, Croteau R. Terpenoid metabolism. *Plant Cell.* 1995;7(7):1015–26. <https://doi.org/10.1105/tpc.7.7.1015>

Additional information

Peer review: Publisher thanks the Sectional Editor and anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information: Available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy.

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting.

Copyright: © The Author(s). This is an open-access article distributed under the Creative Commons Attribution License CC BY 4.0.

Publisher information: Plant Science Today is published by Horizon e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.