JUATIKA
JURNAL AGRONOMI TANAMAN TROPIKA
VOL. 7 NO. 3 September 2025

DOI :https://doi.org/10.36378/juatika.v5i2.4619
eissn 2656-1727
pissn 2684-785X
pages : 801 – 810

RESEARCH ARTICLE Open Access

Allelopathic Effects of *Kyllinga brevifolia*, *Eleusine indica*, and *Sphagneticola trilobata* on Lettuce (*Lactuca sativa*)

Dang Duy Tan[1,*], Irawati Chaniago[1], Aprizal Zainal[1]

Abstract

Weeds are a significant constraint to crop production as they compete for vital resources and release allelochemicals that may hinder the growth of surrounding plants. This study investigated the allelopathic effects of plant litter from *Kyllinga brevifolia*, *Eleusine indica*, and *Sphagneticola trilobata* on the germination and early development of *Lactuca sativa*. Two bioassays were conducted: a sandwich method in the laboratory and a pot experiment in a greenhouse. Dried weed residues were applied at concentrations of 1%, 2%, 3%, and 4% to evaluate their impact on seed germination, root and shoot elongation, and biomass accumulation in lettuce. The results showed that all three species inhibited seed germination and seedling growth in a concentration-dependent manner. In the sandwich assay, germination rates at 4% residue concentration declined to 58.89% (*K. brevifolia*), 76.67% (*E. indica*), and 46.67% (*S. trilobata*), compared to 96.67% in the control. The strongest inhibition of radicle growth was observed in *K. brevifolia* (91.60%). In the pot experiment, both *K. brevifolia* and *S. trilobata* consistently reduced all measured growth parameters. At 4%, shoot length declined to 9.18 mm and 5.42 mm, and dry shoot weight decreased to 2.90 mg and 3.09 mg, respectively. In contrast, *E. indica* slightly increased shoot biomass at moderate concentrations, suggesting a potential stimulatory effect. These findings demonstrate that residues from these weeds possess strong allelopathic potential and could be explored as natural agents for environmentally friendly weed management.

Keywords: Plant Growth Inhibition, Pot Experiment, Sandwich Method, Seed Germination, Weed Interference

1. Introduction

In agroecological systems, weed infestation can directly impair crop development, resulting the reduction in crop yield. Weeds and crops compete for resources such as water, nutrients, and light. In this competition, weeds gain an advantage when their evolution allows them to outcompete agricultural plants (Sutherland, 2004). Moreover, weeds often act as a secondary host for phytopathogens, including insects and pathogens that can infect crops (Alluri & Saha, 2024; Rasul et al., 2024). In addition to directly affecting crops through competition, various weeds can indirectly interact with crops by releasing secondary metabolites, known as allelochemicals, into the environment. This phenomenon, called allelopathy, refers to the positive or negative effects that one plant has on another through these chemical releases (Semmar, 2024; Song et al., 2024). Allelochemicals, released from seeds or seedling roots through processes like exudation, leaching, or decay, can last in soil even after plant death. These compounds often reduce germination percentage and suppress the growth of neighboring plants (Arora et al., 2024; El-Masry et al., 2019; Hussain et al., 2020; Imad et al., 2021).

The study of allelopathy and allelochemical effects including their release into the environment, interactions with plants, and effects on soils is a focus in allelopathy research. The sandwich method is a simple and rapid bioassay technique in which dried plant material is embedded between layers of agar to assess its potential phytotoxicity. This method allows quick detection of allelopathic activity by observing inhibitory effects on seed germination and seedling growth, and is suitable for screening a large number of samples, especially from leaf litter leachates (Fujii et al., 2003). To complement laboratory experimental approaches, pot experiments provide a more realistic soil-based culture setting that

*Correspondence: 2320248001_dang@student.unand.ac.id
1) Universitas Andalas - Limau Manis, Kec. Pauh, Kota Padang, Sumatera Barat 25175, Indonesia

mimics natural conditions occurring in the field, allowing the assessment of allelopathy interactions in a more ecologically relevant context (Khatri et al., 2024; Tian et al., 2022). Among the tested species, *Lactuca sativa* (lettuce) is widely used due to its high sensitivity to a wide range of allelopathy, rapid germination, and uniform growth, making it a reliable bioindicator for assessing heterosis effects (Hussain et al., 2020; Mukaromah et al., 2017; Rashid et al., 2010).

In Indonesia, several invasive weeds including *Kyllinga brevifolia*, *Eleusine indica*, and *Sphagneticola trilobata* are known to interfere with crop productivity. *K. brevifolia* is notable for its adaptability and widespread presence in agricultural areas (Rodiyati & Nakagoshi, 2003). *E. indica*, commonly found in Indonesian oil palm plantations, has developed resistance to glyphosate herbicide, complicating its management (Kurniadie et al., 2023). *S. trilobata* is recognized as a noxious weed in agricultural lands and along roadsides, further disrupting farming activities (Handayani et al., 2021).

Although several studies have investigated the allelopathic properties of these species (*K. brevifolia*, *E. indica*, and *S. trilobata*) systematic and comparative evaluations under controlled conditions remain limited (Kawabata et al., 1994; Perera et al., 2023; Rahardiyan et al., 2019). This study was therefore conducted to assess the allelopathic effects of *K. brevifolia*, E. *indica*, and *S. trilobata* using the sandwich method and pot experiments, aiming to enhance understanding of their ecological roles and support the development of sustainable weed management strategies.

2. Material and Methods

2.1. Plant Materials

Fresh above-ground parts (i.e., shoots with flowers and leaves) of weed samples were collected from the outdoor experimental area of Andalas University campus, Padang (0°55'24.3"S 100°27'17.0"E) altitude of ± 255 m above the sea level. Plant samples were air-dried at room temperature, then ground to fine powder and stored in sealed plastic bag until use. For test plant, *L. sativa* (lettuce) was used because lettuce is reliable plant to investigate the inhibitory and stimulatory allelochemicals at low concentrations (Fujii, 1990). Lettuce seed were bought at local store (Grand Rapids-PT. East West Seed Indonesia).

2.2. Laboratory Experiment

The allelopathic effects of weeds powder were studied using the sandwich method developed by Fujii (2003) with modifications to suit the characteristics of the plant materials. The concentration levels were adapted from the method proposed by Koodkaew and Rottasa (2017).

Agar powder (bacteriological grade) was prepared as a 0.5% (w/v) solution and autoclaved at 121°C for 15 minutes. After autoclaving, the agar solution was cooled to approximately 45°C. Two mL of the agar solution was added into each well of six-well, multi-dish plastic plates (85.4 × 127.6 mm). Once the first agar layer solidified, leaf samples were placed on the agar surface in each well according to the treatment (1%, 2%, 3%, 4%) design shown in **Table 1**. Then, 1 mL of agar solution was carefully added to the leaf samples, forming the middle layer. After this layer solidified, a final 2 mL layer of agar was added on top and allowed to gelatinization at room temperature for 30–60 minutes. This setup forms a three-layer agar medium, with the plant sample embedded between two agar layers (2 mL bottom, 1 mL middle, and 2 mL top). Five seeds of lettuce were placed on the surface of the top agar layer in each well. The plates were then covered with plastic lids and incubated in the dark in a tissue culture room maintained at a stable room temperature (~25°C). Controls were prepared using the same procedure but without the addition of plant samples between the agar layers. After 5 days (120 hours), the length of the radicle and hypocotyl was measured (12 seedlings per replication), and the germination percentage was recorded. Each treatment was conducted with three replications, with each replication consisting of one six-well multi-dish plastic plate.

2.3. Greenhouse Experiment

The allelopathic effects of weed powder were studied using a pot experiment method developed by Koodkaew and Rottasa (2017) with modifications to suit local experimental conditions. Soil was collected from a relatively weed-free site in Padang to minimize potential interference from phenolic compounds naturally released by decomposing vegetation. No fertilizers, pesticides, or herbicides were applied during the experiment.

The collected soil was air-dried, crushed, and thoroughly mixed with weed powder at concentrations of 1%, 2%, 3%, and 4% (**Table 1**). The control treatment was prepared similarly but without the addition of any plant material. Each pot got 2 kg of the treated soil mixture. The mixtures were placed into square-bottomed plastic pots (15 × 15 cm base, 20 cm height), and ten lettuce seeds were sown per pot at a depth of approximately 2 cm. Pots were maintained under adequate watering conditions in a controlled environment for 31 days. After 31 days, plants were carefully uprooted and washed with water.

Growth parameters including shoot length, root length, and both fresh and dry weights were measured using three selected lettuce plants per replication. Shoot length, root length and fresh weights were recorded immediately after harvesting. Samples were then oven-dried at 70°C for 72 hours, and dry weights were subsequently measured. Each treatment consisted of four replications, with each replication represented by one plastic pot containing ten lettuce seeds. The work flow of these experiment is

presented in **Figure 1.**

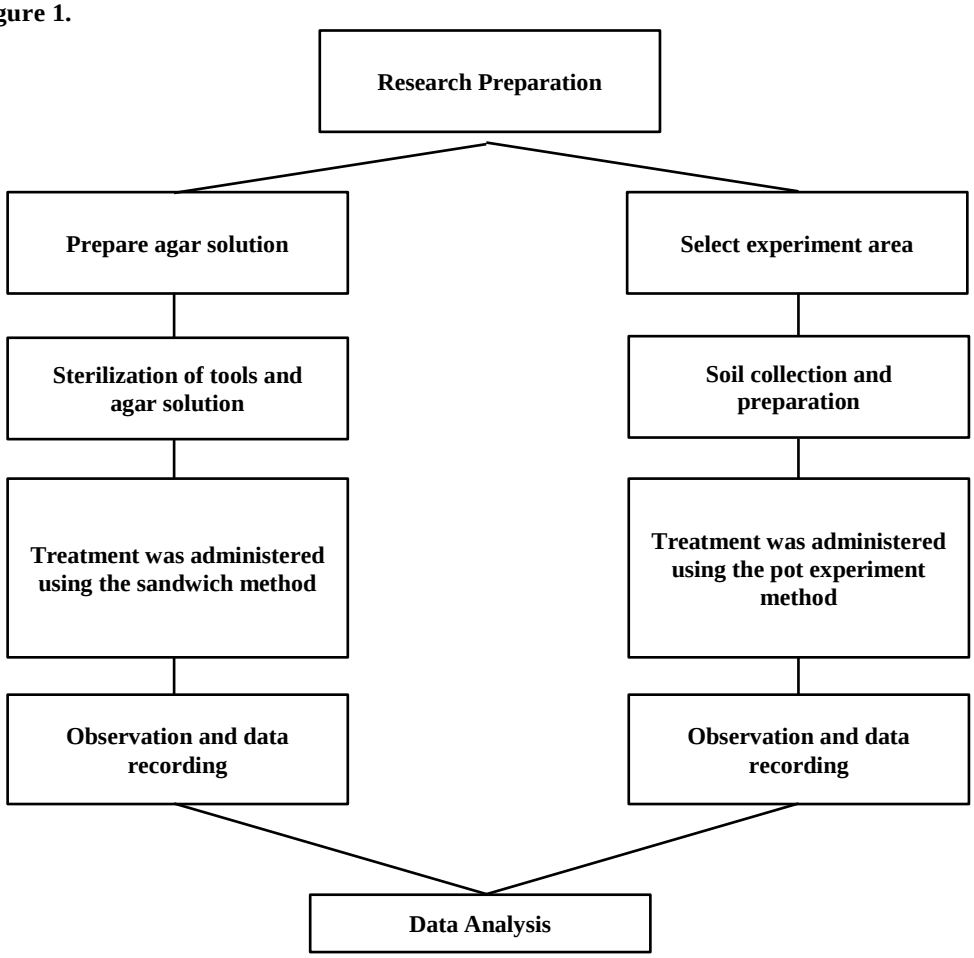

Figure 1. Research flow diagram

Table 1: Residue concentrations of plant sample applied to lettuce

Plant sample (g)		Equivalent concentration (%)
Laboratory experiment (agar volume 5 mL)	Greenhouse experiment (soil amount 2 kg)	
0.05	20	1%
0.10	40	2%
0.15	60	3%
0.20	80	4%

2.4. Data analysis

The sandwich method was conducted using a randomized complete block design (RCBD) with three replications, performed sequentially: the second replication was initiated after the completion of the first, and the third followed thereafter. In contrast, the pot experiment was also arranged in an RCBD but all four replications were carried out simultaneously under uniform outdoor conditions.

The data were analyzed using ANOVA, and differences were considered statistically significant when $P < 0.05$. Differences between treatments were determined using Duncan's multiple range test (DMRT) in IBM SPSS® Statistics version 22.

3. Results and Discussion

3.1. Laboratory experiment

3.1.1. Germination percentage

The germination percentage of *L. sativa* seeds was significantly affected by the application of increasing concentrations of plant litter from *K. brevifolia*, *E. indica*, and *S. trilobata* (**Figure 2**). In all three species, the control (0%) showed the highest germination rate at 96.67%. For *K. brevifolia*, germination decreased with increasing litter concentration, with a significant reduction observed at 3% (67.78%) and the lowest at 4% (58.89%). Similar trends were observed for *E. indica*, although the decline was more gradual, with germination slightly decreasing from 85.56% at 1% to 76.67% at 4%. The most allelopathic effect was recorded in *S. trilobata*, where germination dropped to 46.67% at the 4% concentration.

Concentration-dependent inhibition is one of the fundamental characteristics of allelopathic responses, in which higher amounts of released compounds can lead to stronger germination suppression (Li et al., 2021; Talhi et al., 2020; Zhao et al., 2022). The observed decline in *L.*

sativa germination with increasing litter concentrations indicates the presence of allelopathic potential in *K. brevifolia*, *E. indica*, and *S. trilobata*. Not only affect the germination of *L. sativa*, another result showed that when at high concentrations, *S. trilobata* extract also recude the germination percentage of *Amaranthus cruentus* (Jose & Shaji, 2020).

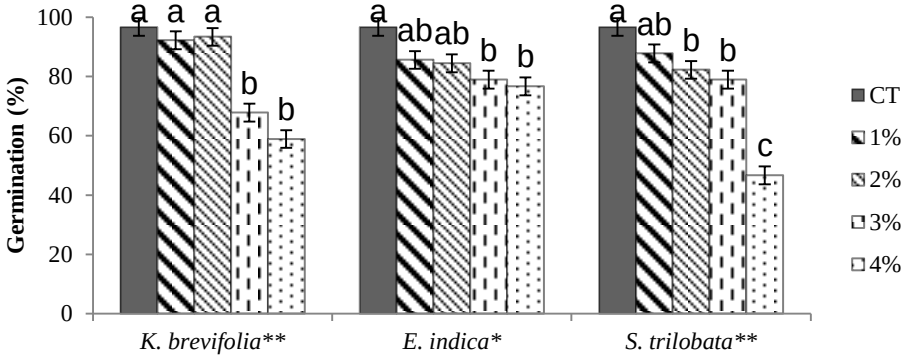

Figure 2. Allelopathic effects of plant litter from *K. brevifolia*, *E. indica*, and *S. trilobata* litter on germination percentage of *L. sativa* using modified sandwich method.

Different lowercase letters (a, b, c, etc.) indicate significant differences among concentration treatments (CT (0%), 1%, 2%, 3%, and 4%) within the same plant species at $\alpha = 0.05$, as determined by Duncan's Multiple Range Test (DMRT). Asterisks indicate the level of statistical significance between treatments: ** = significant at 1% level ($p < 0.01$); * = significant at 5% level ($p < 0.05$).

3.1.2. Hypocotyl elongation

Hypocotyl elongation of *L. sativa* seedlings was significantly inhibited by increasing concentrations of *K. brevifolia*, *E. indica*, and *S. trilobata* litter (**Figure 3, 4**). In the control treatment, the average hypocotyl length was 26.58 mm. As litter concentration increased, hypocotyl growth consistently declined. For *K. brevifolia*, hypocotyl length decreased from 20.84 mm at 1% to 14.14 mm at 4%, corresponding to inhibition rates of 21.60% at 1% and 46.78% at 4%. *E. indica* showed a steady reduction in hypocotyl elongation, from 18.20 mm at 1% to just 6.79 mm at 4%, resulting in inhibition percentages of 31.52% at 1% and 74.45% at 4%. *S. trilobata* showed the light effect at 1% (25.50 mm; 4.06% inhibition), but inhibition increased at higher concentrations to 57.66% at 4%, with hypocotyl length reduced to 11.25 mm.

The hypocotyl elongation of *L. sativa* seedlings was significantly reduced with increasing concentrations of litter from *K. brevifolia*, *E. indica*, and *S. trilobata*, suggesting the presence of phytotoxic compounds that disrupt early seedling development. The decline in hypocotyl length shows a typical dose-dependent response commonly associated with allelopathic interactions (Ishak et al., 2021; Sothearith et al., 2021). This kind of inhibition is not limited to the sandwich bioassay; similar trends have been widely reported in other allelopathy studies using different methods, such as aqueous extracts or soil amendment approaches, further supporting the role of allelochemicals in growth suppression (Arroussi et al., 2022; Pannacci et al., 2020).

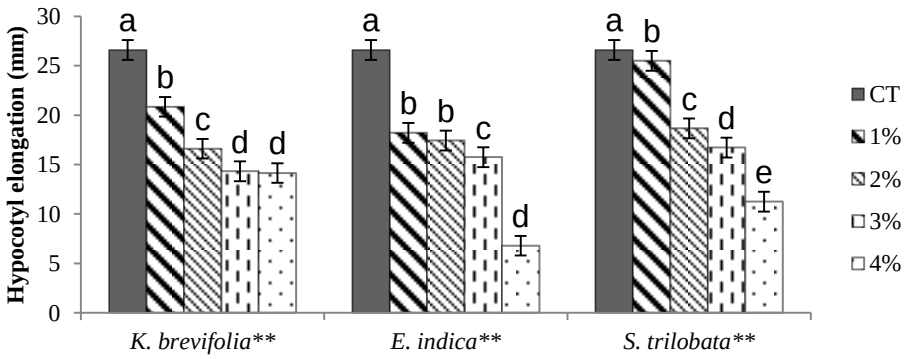

Figure 3. Allelopathic effects of plant litter from *K. brevifolia*, *E. indica*, and *S. trilobata* litter on hypocotyl elongation of *L. sativa* using modified sandwich method.

Different lowercase letters (a, b, c, etc.) indicate significant differences among concentration treatments (CT (0%), 1%, 2%, 3%, and 4%) within the same plant species at $\alpha = 0.05$, as determined by Duncan's Multiple Range

Test (DMRT). Asterisks indicate the level of statistical significance between treatments: ** = significant at 1% level ($p < 0.01$).

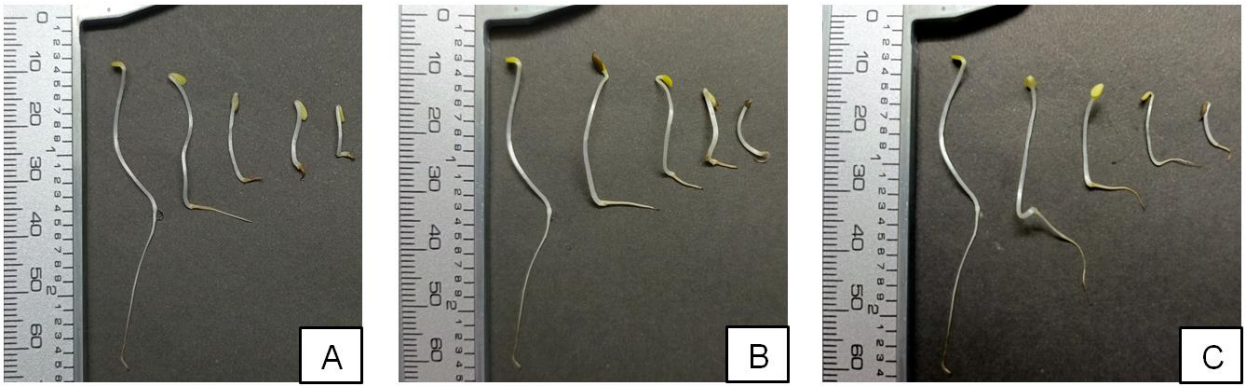

Figure 4. Allelopathic effects of plant litter from *K. brevifolia* (A), *E. indica* (B), and *S. trilobata* (C) on the elongation of hypocotyls and radicles in *L. sativa*, assessed using a modified sandwich method. From left to right, the applied concentrations were 0% (Control), 1%, 2%, 3%, and 4%.

3.1.3. Radicle elongation

The elongation of radicles in *L. sativa* seedlings was reduced in response to increasing concentrations of *K. brevifolia*, *E. indica*, and *S. trilobata* litter (**Figure 4, 5**). At control treatment, seedlings exhibited an average radicle length of 31.05 mm. Exposure to *K. brevifolia* resulted in a steep and progressive decline in radicle elongation, with measurements dropping from 11.51 mm at 1% to 2.61 mm at 4% corresponding to inhibition rates are 62.93% at 1% and 91.60% at 4%, respectively. Similar phenomenon was seen with *E. indica*, where increasing concentrations led to lengths of radicle from 6.92 mm (1%) to 3.07 mm (4%), resulting in inhibition rates of 77.72% at 1% and 90.11% at 4%. In the case of *S. trilobata*, the reduction was more moderate at lower concentrations, but still substantial at higher ones. Radicles measured 16.28 mm at 1% to 3.46 mm at 4%, reflecting inhibition percentages of 47.58% at 1% to at 4%, respectively.

The reduction in radicle elongation of *L. sativa* seedlings in response to increasing concentrations of *K. brevifolia*, *E. indica*, and *S. trilobata* litter provides strong evidence of allelopathic activity in all three species. As the first organ to emerge during germination and the primary interface with the surrounding substrate, the radicle is particularly vulnerable to phytotoxic compounds. Its direct exposure to allelochemicals makes it a sensitive and reliable indicator of allelopathic stress. This observation is consistent with previous studies showing that allelochemicals can inhibit root cell division and elongation processes that are often more affected in roots than in shoots or hypocotyls due to this direct contact with toxic residues, a screening experiment have a similar result about *E. indica* (Ain et al., 2023; Ishak et al., 2021).

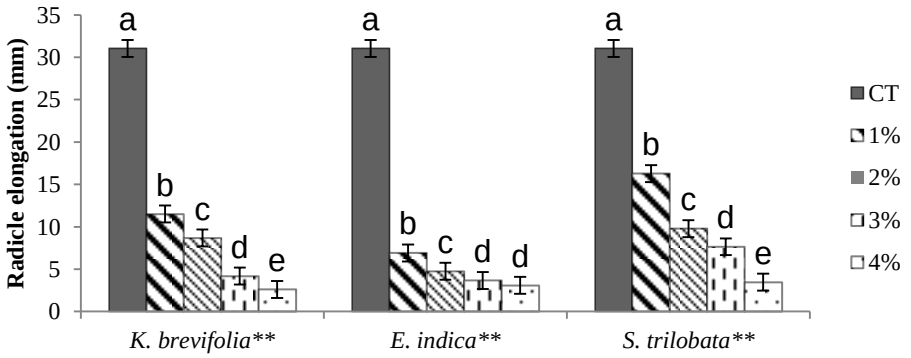

Figure 5: Allelopathic effects of *K. brevifolia*, *E. indica*, and *S. trilobata* litter on radicle elongation of *L. sativa* using modified sandwich method. Different lowercase letters (a, b, c, etc.) indicate significant differences among concentration treatments (CT (0%), 1%, 2%, 3%, and 4%) within the same plant species at $\alpha = 0.05$, as determined by Duncan's Multiple Range Test (DMRT). Asterisks indicate the level of statistical significance between treatments: ** = significant at 1% level ($p < 0.01$).

3.2. Greenhouse experiment

3.2.1. Allelopathic effects of *K. brevifolia*

The application of *K. brevifolia* litter significantly affected the growth of *L. sativa* seedlings in terms of shoot

and root length, as well as fresh and dry biomass (**Table 2, Figure 6**). The biological indicators of the plants decreased as the concentration of litter increased. At the highest treatment level (4%), shoot and root lengths were reduced to 9.18 mm and 54.11 mm. Similarly, fresh weight of shoots declined drastically from 151.43 mg in the control to 24.39 mg at 4%, while root fresh weight decreased from 29.24 mg to 3.62 mg, indicating strong inhibitory effects on biomass accumulation. Dry weight followed the same trend, with shoot dry weight dropping from 14.68 mg to 2.90 mg, and root dry weight from 5.28 mg to 1.08 mg across the treatment gradient.

Figure 6. Allelopathic effects of *K. brevifolia* litter on *L. sativa* growth in greenhouse experiment. From left to right, the applied concentrations were 0% (Control), 1%, 2%, 3%, and 4%.

The reduction in all growth parameters of *L. sativa* seedlings following the application of *K. brevifolia* litter confirms the strong allelopathic potential of this species under soil conditions. The consistent and concentration-dependent decline in shoot and root elongation, along with significant reductions in fresh and dry biomass, suggests that *K. brevifolia* residues release allelochemical compounds that affect the early seedling development. These inhibitory effects may result not only from direct allelochemical activity but also from indirect alterations to soil properties and microbial communities present in the soil, which can further intensify the severity of allelopathic interactions (Bonanomi et al., 2021; Schandry & Becker, 2020). Previous studies have shown that *K. brevifolia* contains a variety of allelochemicals, including phenolics and terpenoids, which are known for their phytotoxic properties, a study showed that the increase of planting densities of *K. brevifolia* reduced *Cynodon dactylon* (bermudagrass) growth (Babu et al., 2025; Kawabata et al., 1994). These compounds may be released into the soil during the decomposition of plant litter, allowing allelochemicals to accumulate and affect to the neighboring plants.

3.2.2. Allelopathic effects of *E. indica*

Treatment with *E. indica* litter led to variable effects on *L. sativa* growth (**Table 3; Figure 7**). While certain parameters showed inhibitory patterns, others displayed a stimulatory response at specific concentrations. Shoot length varied inconsistently with treatment levels. Although the control group recorded 21.37 mm, the highest value (22.33 mm) was observed at 1%, followed by a decline to 16.42 mm and 16.97 mm at 2% and 3%, respectively, before slightly rising to 19.71 mm at 4%. Root length followed a similar non-linear response, decreasing from 130.63 mm (control) to 95.81 mm (2%), then increasing again to 117.86 mm at 4%. Shoot fresh weight increased at several concentrations compared to the control. The highest value (158.59 mg) was observed at 2%, while 4% also maintained a high biomass (156.30 mg), in contrast to 151.43 mg in the control. Root fresh weight, however, exhibited a dose-dependent increase, from 8.34 mg (1%) to 16.62 mg (4%). Dry weights of shoots and roots reflected a similar situation. Shoot dry weight peaked at 14.55 mg at 2%, then declined gradually, whereas root dry weight steadily increased from 3.12 mg (1%) to 3.57 mg (4%).

Table 2: Effects of *K. brevifolia* litter on *L. sativa* growth in greenhouse experiment

Concentration (%)	Length (mm)		Fresh weight (mg)		Dry weight (mg)	
	Shoot	Root	Shoot	Root	Shoot	Root
CT (0%)	21.37 ± 1.42 a	130.63 ± 3.32 a	151.43 ± 6.87 a	29.24 ± 1.37 a	14.68 ± 0.85 a	5.28 ± 0.24 a
1%	17.09 ± 1.10 b	88.88 ± 8.56b	95.72 ± 6.61 b	10.43 ± 0.53 b	9.76 ± 0.33 b	2.92 ± 0.27 b
2%	12.24 ± 1.11 c	79.39 ± 5.96 c	52.88 ± 3.69 c	5.95 ± 0.34 c	4.69 ± 0.46 c	1.44 ± 0.09 c
3%	9.04 ± 0.53 d	74.94 ± 4.51 c	40.85 ± 5.24 d	5.65 ± 0.30 c	4.37 ± 0.20 c	1.45 ± 0.10 c
4%	9.18 ± 0.58 d	54.11 ± 5.30 d	24.39 ± 1.33 e	3.62 ± 0.28 d	2.90 ± 0.27 d	1.08 ± 0.12 d
Sig.	**	**	**	**	**	**

Different lowercase letters within each column indicate significant differences among concentrations (0% (Control), 1%, 2%, 3%, and 4%) of *K. brevifolia* litter, as determined by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. Asterisks (**) denote significant differences at the 1% level ($p < 0.01$).

Figure 7. Allelopathic effects of *E. indica* litter on *L. sativa* growth in greenhouse experiment. From left to right, the applied concentrations were 0% (Control), 1%, 2%, 3%, and 4%.

Treatment with *E. indica* litter produced variable and non-linear effects on *L. sativa* growth, indicating a more complex allelopathic response. Some parameters, such as root length, showed clear inhibitory trends at certain concentrations, while others, particularly shoot biomass, exhibited a mild stimulatory effect. Shoot fresh and dry weights increased slightly at moderate concentrations, surpassing those of the control group. Similarly, root biomass displayed a gradual upward trend with increasing residue levels. This stimulatory effect has been reported in allelopathy studies when low concentrations of certain allelochemicals act as growth enhancers before becoming toxic at higher doses (Cheng & Cheng, 2015; Xuan et al., 2005). Moreover, plant residues with allelopathic potential, when mixed into the soil, may affect seed germination and seedling development not only by releasing phytotoxic compounds directly, but also through their influence on soil environmental factors. These influences can include changes in moisture retention, soil temperature, and the stimulation of microbial activity, all of which can interact with the organic matter to enhance the release or transformation of allelochemicals (Inderjit, 2001; Scavo et al., 2019; Teasdale & Mohler, 2000). In the case of *E. indica*, the rigid and fibrous tissue structure, which is rich in lignin and cellulose, decomposes slowly and contributes to the formation of an organic layer that improves soil texture. This physical modification can temporarily improve soil aeration and water infiltration, thereby facilitating root development in some plant species (Fu et al., 2024; Russell, 2014). However, during the decomposition process, residues of *E. indica* may release phenolic acids and other phytotoxic compounds that interfere with seed germination and root elongation (Kashyap et al., 2023; Phương et al., 2017; Tantiado & Saylo, 2012; Valentino et al., 2018).

Table 3: Effects of *E. indica* litter on *L. sativa* growth in greenhouse experiment

Concentration (%)	Length (mm)		Fresh weight (mg)		Dry weight (mg)	
	Shoot	Root	Shoot	Root	Shoot	Root
CT (0%)	21.37 ± 1.42 ab	130.63 ± 3.32 a	151.43 ± 6.87 a	29.24 ± 1.37 a	14.68 ± 0.85 a	5.28 ± 0.24 a
1%	22.33 ± 1.15 a	107.65 ± 3.95 c	115.19 ± 6.73 b	8.34 ± 0.61 e	10.59 ± 0.79 d	3.12 ± 0.16 bc
2%	16.42 ± 1.39 c	95.81 ± 6.08 d	158.59 ± 7.65 a	10.98 ± 0.58 d	14.55 ± 1.12 ab	3.06 ± 0.27 c
3%	16.97 ± 2.08 c	109.82 ± 4.61 bc	120.08 ± 3.21 b	13.76 ± 1.01 c	12.05 ± 1.16 cd	3.20 ± 0.21 bc
4%	19.71 ± 0.91 b	117.86 ± 7.37 b	156.30 ± 13.80 a	16.62 ± 0.95 b	13.01 ± 0.93 bc	3.57 ± 0.52 b
Sig.	**	**	**	**	**	**

Different lowercase letters within each column indicate significant differences among concentrations (0% (Control), 1%, 2%, 3%, and 4%) of *E. indica* litter, as determined by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. Asterisks (**) denote significant differences at the 1% level ($p < 0.01$).

3.2.3. Allelopathic effects of *S. trilobata*

Application of *S. trilobata* litter caused a steady decline in all measured growth parameters of *L. sativa*. Shoot and root elongation, as well as biomass, decreased clearly as litter concentration increased, indicating strong inhibitory potential (**Table 4; Figure 8**). Shoot length decreased progressively from 21.37 mm in the control to 17.12 mm at 1%, followed by drastic declines to 8.36 mm (2%), 6.79 mm (3%), and 5.42 mm (4%). Root length dropped from 130.63 mm in untreated soil to 98.03 mm at 1%, then fell further to 46.42 mm at 4%, showing a cumulative inhibition effect with increasing concentration. Shoot fresh weight was reduced by nearly half at 1% (80.62 mg) and declined further to 20.01 mg at 4%, compared to 151.43 mg in the control. Root fresh weight decreasing from 29.24 mg (control) to 13.79 mg (1%) and only 2.28 mg at 4%.The dry weight of shoots reduced from 14.68 mg in the control to 7.12 mg at 1%, and dropping to just 3.09 mg at 4%. Similar, root dry weight reduced from 5.28 mg to 1.16 mg across treatments.

The consistent, concentration-dependent inhibition of all measured growth parameters in *L. sativa* seedlings following the application of *S. trilobata* litter underscores the strong allelopathic potential of this invasive species. The progressive reduction in shoot and root elongation,

coupled with marked declines in both fresh and dry biomass, suggests that *S. trilobata* releases allelochemical compounds capable of severely impairing early seedling development. *S. trilobata*, characterized by its vigorous growth and soft, rapidly decomposing tissues, produces various allelochemicals including phenolics, flavonoids, and saponins, that can interfere with key physiological processes in neighboring plants. For example, treatment with *S. trilobata* aqueous extract or compost soil mixture significantly reduced seed germination, shoot height, growth rate, leaf area, biomass (fresh and dry), root length, pod length, and yield of *Phaseolus vulgaris* (Araújo et al., 2021; Mến et al., 2019; Perera et al., 2023). Due to its rapid growth and high biomass turnover, *S. trilobata* can induce substantial allelopathic pressure, potentially influencing plant community composition and dynamics in both natural ecosystems and cultivated lands (Qi et al., 2014).

Figure 8. Allelopathic effects of *S. trilobata* litter on *L. sativa* growth in greenhouse experiment. From left to right, the applied concentrations were 0% (Control), 1%, 2%, 3%, and 4%.

Table 4: Effects of *S. trilobata* litter on *L. sativa* growth in Greenhouse experiment

Concentration (%)	Length (mm)		Fresh weight (mg)		Dry weight (mg)	
	Shoot	Root	Shoot	Root	Shoot	Root
CT (0%)	21.37 ± 1.42 a	130.63 ± 3.32 a	151.43 ± 6.87 a	29.24 ± 1.37 a	14.68 ± 0.85 a	5.28 ± 0.24 a
1%	17.12 ± 0.72 b	98.03 ± 5.18 b	80.62 ± 4.43 b	13.79 ± 0.64 b	7.12 ± 0.48 b	2.13 ± 0.11 b
2%	8.36 ± 0.45 c	71.70 ± 5.50 c	43.25 ± 5.09 c	4.31 ± 0.27 c	5.38 ± 0.39 c	1.38 ± 0.13 c
3%	6.79 ± 0.68 d	55.34 ± 5.60 d	26.89 ± 1.78 d	2.50 ± 0.28 d	3.23 ± 0.15 d	1.14 ± 0.08 c
4%	5.42 ± 0.46 d	46.42 ± 6.11 e	20.01 ± 2.70 d	2.28 ± 0.34 d	3.09 ± 0.11 d	1.16 ± 0.13 c
Sig.	**	**	**	**	**	**

Different lowercase letters within each column indicate significant differences among concentrations (0% (Control), 1%, 2%, 3%, and 4%) of *S. trilobata* litter, as determined by Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$. Asterisks (**) denote significant differences at the 1% level ($p < 0.01$).

4. Conclusion

The findings of this study provide clear evidence that *Kyllinga brevifolia*, *Eleusine indica*, and *Sphagneticola trilobata* possess significant allelopathic activity that can negatively affect seed germination and early growth of *Lactuca sativa*. Both the sandwich bioassay and pot experiment showed the inhibition effect to the germination and growth of the test plant, especially at higher residue concentrations. Further studies are necessary to isolate the active compounds, determine their mechanisms of action, and assess their environmental persistence and effectiveness under field conditions before practical application.

References

Ain, Q., Mushtaq, W., Shadab, M., & Siddiqui, M. B. (2023). Allelopathy: An alternative tool for sustainable agriculture. *Physiology and Molecular Biology of Plants, 29*(4), 495–511. https://doi.org/10.1007/s12298-023-01305-9

Alluri, G., & Saha, D. (2024). Emerging perspectives on non-chemical weed management tactics in container ornamental production in the United States. *Horticulturae, 10*(3). https://doi.org/10.3390/horticulturae10030281

Araújo, C. A., Morgado, C. S. A., Gomes, A. K. C., Gomes, A. C. C., & Simas, N. K. (2021). *Asteraceae* family: A review of its allelopathic potential and the case of *Acmella oleracea* and *Sphagneticola trilobata*. *Rodriguesia, 72*. https://doi.org/10.1590/2175-7860202172137

Arora, S., Husain, T., & Prasad, S. M. (2024). Allelochemicals as biocontrol agents: Promising aspects, challenges and opportunities. *South African Journal of Botany, 166*, 503–511. https://doi.org/10.1016/j.sajb.2024.01.029

Arroussi, J., Ouerfelli, M., Smaoui, A., Ahmed, H. Ben, Kaâb, S. Ben, & Kaâb, L. B. Ben. (2022). Antioxidant activity of seven plant extracts collected from Tunisia and their allelopathic potential on *Lactuca sativa* L. and *Phalaris minor* L. *South African Journal of Botany, 148*, 135–143. https://doi.org/10.1016/j.sajb.2022.04.029

Babu, M., Abirami, T., Velu, K., Janaki, A., & Fahd, A. (2025). Larvicidal activity and phytochemical composition of *Kyllinga brevifolia* (Poales: Cyperaceae) against mosquito vectors. *Journal of the Kansas Entomological Society, 98*(2), 62–74.

Bonanomi, G., Zotti, M., Idbella, M., Mazzoleni, S., & Abd-ElGawad, A. M. (2021). Microbiota modulation of allelopathy depends on litter chemistry: Mitigation or exacerbation? *Science of the Total Environment, 776*, 145942. https://doi.org/10.1016/j.scitotenv.2021.145942

Cheng, F., & Cheng, Z. (2015). Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in Plant Science, 6*, 1020. https://doi.org/10.3389/fpls.2015.01020

El-Masry, R. R., Ahmed, S. A. A., El-Rokiek, K. G., Messiha, N. K., & Mohamed, S. A. (2019). Allelopathic activity of the leaf powder of *Ficus nitida* on the growth and yield of *Vicia faba* and associated weeds. *Bulletin of the National Research Centre, 43*, 1–7. https://doi.org/10.1186/s42269-019-0114-x

Fu, F., Luo, J., Zhao, L., Yang, F., & Wang, N. (2024). Impact of cellulose and lignin on restoration of vegetation and soil chemical properties for saline-alkali soil of Songnen Plain. *PLoS ONE, 19*(1), e0296366. https://doi.org/10.1371/journal.pone.0296366

Fujii, Y. (1990). Survey of Japanese weeds and crops for the detection of water-extractable allelopathic chemicals using Richards' function fitted to lettuce germination test. *Weed Research Japan, 35*, 362–370.

Fujii, Y., Parvez, S. S., Parvez, M. M., Ohmae, Y., & Iida, O. (2003). Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. *Weed Biology and Management, 3*(4), 233–241.

Handayani, A., Junaedi, D. I., & Zuhud, E. A. M. (2021). Ecological risk assessment of potentially invasive alien plant species in Cibodas Biosphere Reserve, West Java, Indonesia. *IOP Conference Series: Earth and Environmental Science, 914*(1), 012035. https://doi.org/10.1088/1755-1315/914/1/012035

Hussain, M. I., El-Sheikh, M. A., & Reigosa, M. J. (2020). Allelopathic potential of aqueous extract from *Acacia melanoxylon* R. Br. on *Lactuca sativa*. *Plants, 9*(9), 1228. https://doi.org/10.3390/plants9091228

Imad, M., Idrees, M., Hadi, F., Memon, N. H., & Zhang, Z. (2021). Allelopathic effect of *Parthenium hysterophorus* extract on seed germination and seedling growth of selected plants. *Pakistan Journal of Botany, 53*(6), 2187–2197. https://doi.org/10.30848/PJB2021-6(9)

Inderjit. (2001). Soil: Environmental effects on allelochemical activity. *Agronomy Journal, 93*(1), 79–84.

Ishak, M. S., Ain, M. N., Sahid, I., & Mardiana-Jansar, K. (2021). Allelopathic screening of Malaysian noxious weeds and several medicinal plants as potential alleloherbicides. *Journal of Environmental Biology, 42*, 762–774.

Jose, A. M., & Shaji, A. G. (2020). Allelopathic effects of aqueous leaf extracts of two invasive plants (*Chromolaena odorata* (L.) R. M. King & H. Rob. and *Sphagneticola trilobata* (L.) Pruski) on seed germination of *Amaranthus cruentus* L. *Journal of Advanced Scientific Research, 11*(4), 198–201. http://www.sciensage.info/journal/1359303580JASR_3006121.pdf

Kashyap, P., Shikha, D., Gautam, S., & Rani, U. (2023). Harvesting food from weeds. [Reference incomplete—perlu detail jurnal/buku].

Kawabata, O., Nishimoto, R. K., & Shih, T. C. (1994). Interference of two *Kyllinga* species (*Kyllinga nemoralis* and *Kyllinga brevifolia*) on bermudagrass (*Cynodon dactylon*) growth. *Weed Technology, 8*(1), 83–86. https://doi.org/10.1017/s0890037x00039245

Khatri, K., Negi, B., Bargali, K., & Bargali, S. S. (2024). Toxicological assessment of invasive *Ageratina adenophora* on germination and growth efficiency of native tree and crop species of Kumaun Himalaya. *Ecotoxicology*. https://doi.org/10.1007/s10646-024-02768-6

Koodkaew, I., & Rottasa, R. (2017). Allelopathic effects of giant sensitive plant (*Mimosa pigra*) leaf powder on germination and growth of popping pod and purslane. *International Journal of Agriculture and Biology, 19*(5), 1113–1118.

Kurniadie, D., Widianto, R., Umiyati, U., Widayat, D., Nasahi, C., & Budiawan, A. (2023). Management of *Eleusine indica* (L.) Gaertn resistance to glyphosate herbicide in Indonesia. *Agronomy, 13*(6), 1649. https://doi.org/10.3390/agronomy13061649

Li, J., Chen, L., Chen, Q., Miao, Y., Peng, Z., Huang, B., Guo, L., Liu, D., & Du, H. (2021). Allelopathic effect of *Artemisia argyi* on the germination and growth of various weeds. *Scientific Reports, 11*(1), 4303. https://doi.org/10.1038/s41598-021-83752-6

Li, Y., Zeng, R. S., Liao, H., Xu, Q., & Zhou, Y. (2022). Advances in allelopathy of invasive plant *Mikania micrantha* H.B.K. *Plants, 11*(23), 3290. https://doi.org/10.3390/plants11233290

Liu, H., Liu, C., Liu, Y., & Chen, Y. (2019). Allelopathic effects of invasive *Mikania micrantha* H.B.K. on seed germination and seedling growth of four crops. *Ecotoxicology and Environmental Safety, 182*, 109401. https://doi.org/10.1016/j.ecoenv.2019.109401

Macías, F. A., Mejías, F. J. R., & Molinillo, J. M. G. (2019). Recent advances in allelopathy for weed control: From knowledge to applications. *Pest Management Science, 75*(9), 2413–2436. https://doi.org/10.1002/ps.5360

Mahajan, G., Mutti, N. K., & Chauhan, B. S. (2021). Eco-friendly weed management options for sustainable agriculture. *Plants, 10*(12), 2644. https://doi.org/10.3390/plants10122644

Majid, M., Rahman, M., Khan, S., & Khan, M. A. (2020). Allelopathic effects of *Parthenium hysterophorus* on seed germination and seedling growth of *Brassica rapa*. *Sarhad Journal of Agriculture, 36*(2), 660–668.

Mekki, M. (2021). Allelopathic effects of aqueous extracts of *Sonchus oleraceus* L. on germination and seedling growth of *Triticum aestivum* L. and *Hordeum vulgare* L. *Journal of Plant Interactions, 16*(1), 122–129. https://doi.org/10.1080/17429145.2021.1886783

Meyer, G. A., & Schmid, B. (1999). Experimental demonstration of allelopathic effects of *Goldenrod* (*Solidago canadensis*) on the performance of target species. *Journal of Ecology, 87*(3), 361–368.

Mushtaq, W., Ain, Q., Shadab, M., & Siddiqui, M. B. (2021). Allelopathy and allelochemicals as weed management strategies: A review. *Journal of Plant Diseases and Protection, 128*, 1023–1039. https://doi.org/10.1007/s41348-021-00497-9

Nishida, N., Tamotsu, S., Nagata, N., Saito, C., & Sakai, A. (2005). Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of Chemical Ecology, 31*(5), 1187–1203.

Nourimand, M., & Todd, C. D. (2020). Allelopathic plants: A Canadian perspective. *Botany, 98*(10), 589–603. https://doi.org/10.1139/cjb-2020-0032

Olofsdotter, M., Navarez, D., Rebulanan, M., & Streibig, J. C. (1999). Weed-suppressing rice cultivars: Does allelopathy play a role? *Weed Research, 39*(6), 441–454.

Pandey, D. K., Palni, L. M. S., & Joshi, S. C. (2017). Allelopathic plants. 7. *Parthenium hysterophorus* L. *Allelopathy Journal, 42*(2), 123–154.

Qasem, J. R. (2020). Allelopathic potential of *Chenopodium murale* and *Amaranthus retroflexus* and their possible use as bioherbicides. *Allelopathy Journal, 50*(2), 175–190.

Qin, B., Cao, Y., & Wang, G. (2022). Allelopathic effects of invasive *Solidago canadensis* L. on germination and growth of native plants in China. *Ecological Research, 37*(1), 35–44.

Rahnama, P., & Ghadiri, H. (2021). Allelopathic effects of *Eucalyptus camaldulensis* leaf litter on germination and growth of crops and weeds. *Iranian Journal of Plant Physiology, 11*(4), 3513–3522.

Rana, N., & Rana, D. S. (2016). Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. *International Journal of Plant Research, 29*(1), 1–8.

Rice, E. L. (1984). *Allelopathy* (2nd ed.). Academic Press.

Shrestha, A., & Shrestha, S. (2020). Allelopathic potential of invasive plant *Ageratina adenophora* (Spreng.) on germination and growth of maize (*Zea mays* L.). *International Journal of Applied Sciences and Biotechnology, 8*(3), 365–372. https://doi.org/10.3126/ijasbt.v8i3.31264

Singh, H. P., Batish, D. R., & Kohli, R. K. (2003). Allelopathic interactions and allelochemicals: New possibilities for sustainable weed management. *Critical Reviews in Plant Sciences, 22*(3–4), 239–311.

Wu, H., Pratley, J. E., Lemerle, D., & Haig, T. (1999). Crop cultivars with allelopathic capability. *Weed Research, 39*(3), 171–180.

Xuan, T. D., Tawata, S., Khanh, T. D., & Chung, I. M. (2005). Decomposition of allelopathic plants in soil. *Journal of Agronomy and Crop Science, 191*(3), 162–171.

Zhou, Y., Yu, J., & Zhou, D. (2020). Allelopathic effects of invasive plant *Alternanthera philoxeroides* on seed germination and seedling growth of crops. *Allelopathy Journal, 50*(1), 87–96.