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DOSE -DEPENDENT ALLEOPATHIC RESPONSES OF OKRA TO AQUEOUS LEAF EXTRACTS OF COMMON WEEDS

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Abstract:
Allelopathy plays a significant role in crop–weed interactions and offers an eco-friendly alternative to chemical weed management. The present investigation evaluated the allelopathic effects of three weed species, Alternanthera sessilis, Synedrella nodiflora, and Sphagneticola trilobata, on seed germination and early growth of Abelmoschus esculentus (okra) under in vitro and in vivo conditions. Crude aqueous leaf extracts at 10%, 30%, and 50% concentrations were tested using a completely randomized design. The results revealed marked species- and concentration-dependent effects. Alternanthera sessilis significantly inhibited germination and root growth under in vitro conditions, especially at higher concentrations, while no significant effects were observed in vivo. Synedrella nodiflora caused significant inhibition of germination and root elongation in vitro, with one-way ANOVA confirming significant differences in root length ($p = 0.0238$), whereas in vivo treatments showed improved germination and seedling growth. Sphagneticola trilobata exhibited a hormetic response, while germination percentage varied significantly with concentration ($p = 0.001$). In vivo effects showed a positive trend but were not statistically significant ($p > 0.05$).The study concludes that allelopathic effects vary with experimental conditions and highlights the potential use of selected weeds in sustainable agriculture.

Keywords: Allelopathy, *Abelmoschus esculentus*, weeds, in vitro, in vivo.

INTRODUCTION

Plants interact with one another by releasing chemical compounds into their surrounding environment, leading to either positive or negative biological effects on neighboring plants and microorganisms. This phenomenon, known as allelopathy, plays a significant role in plant community structure, biodiversity, succession, and productivity. Allelopathy is defined as the direct or indirect beneficial or harmful influence of one plant on another through the release of chemical substances called allelochemicals. These compounds may be released through leaching, volatilization, root exudation, or decomposition of plant residues. The effect of allelochemicals largely depends on their concentration, which may stimulate or inhibit plant growth.

Allelopathy has gained attention as an eco-friendly and sustainable approach to weed management, as it reduces reliance on synthetic herbicides that cause environmental pollution, weed resistance, and increased production costs. Weeds are a major constraint in agriculture, competing with crops for resources and often releasing phytotoxic chemicals that suppress crop germination and growth. Common allelopathic effects include reduced seed germination, impaired root and shoot growth, and disruption of physiological processes such as photosynthesis and enzyme activity.

Understanding allelopathic interactions offers promising opportunities for natural weed control, improved crop productivity, and sustainable agricultural practices, particularly through the utilization of naturally occurring allelopathic relationships between crops and This study aims to evaluate the allelopathic effects of crude aqueous extracts from three weed species—*Alternanthera sessilis*, *Synedrella nodiflora*, and *Sphagneticola trilobata*—on the seed germination and seedling growth of okra (*Abelmoschus esculentus*). The research will assess these effects under both controlled laboratory (*in vitro*) and natural soil (*in vivo*) conditions. Additionally, it will establish a comparative baseline by evaluating the growth of okra in water medium across both experimental settings.

MATERIALS AND METHODS

The study was conducted in Palakkad district, Kerala, India, using *Abelmoschus esculentus* (L.) Moench as the test crop. Three weed species—*Alternanthera sessilis*, *Synedrella nodiflora*, and *Sphagneticola trilobata*—were evaluated for allelopathic effects. Fresh leaves were collected, washed, air-dried, and used to prepare crude aqueous extracts at 10%, 30%, and 50% concentrations.

Experiments were carried out under in vitro and in vivo conditions using a completely randomized design with three replicates. Germination percentage, root length, and number of leaves were recorded over 15 days. Data were analyzed using one-way analysis of variance (ANOVA), and treatment means were compared at the 5% significance level.

RESULTS

Allelopathic studies of selected weeds on crop

Leaf extracts of *Alternanthera sessilis*, *Synedrella nodiflora*, and *Sphagneticola trilobata* showed inhibitory effects on the germination and growth of *Abelmoschus esculentus*, indicating their allelopathic potential. Higher extract concentrations caused greater inhibition, significantly reducing germination percentage, root length, and number of leaves. The degree of reduction varied among the selected weeds.

Root length, No. of leaves & Germination percentage of *Abelmoschus esculentus* seedling treated with *Alternanthera sessilis* leaf extracts in in vitro.

Alternanthera sessilis leaf extracts showed concentration-dependent allelopathic effects on *Abelmoschus esculentus*. In vitro, increasing extract concentration reduced germination, root growth, and other growth parameters, with strong inhibition at 30% and 50%, while 10% caused delayed but comparable root length to the control. In vivo, germination percentage increased despite the inhibitory effects observed under laboratory conditions.

Table 1 *One-way ANOVA of the effect of* Sphagneticola trilobata *leaf extract concentration on growth parameters of* Abelmoschus esculentus *seedlings (in vitro)*

Variable	Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p)
Root length (cm)	Between Groups	4.62	3	1.54	8.27	0.003*
	Within Groups	2.23	12	0.19		
	Total	6.85	15			

Table 1 the ANOVA results (p = 0.003) confirm a statistically significant difference in the root length of okra seedlings treated with different concentrations of *Sphagneticola trilobata* leaf extract. This indicates that the extract concentration has a meaningful effect on root growth, with higher concentrations likely promoting longer roots compared to the control, as supported by the post-hoc tests.

Table 2 Tukey's HSD post-hoc comparison of different concentrations of *Sphagneticola trilobata* leaf extract on *Abelmoschus esculentus* seedlings (in vitro)(A) Root length (cm)

Concentration (I)	Concentration (J)	Mean Difference (I–J)	Std. Error	Sig.
Control	10%	0.21	0.08	0.041*
Control	30%	0.48	0.09	0.003*
Control	50%	0.72	0.10	0.001*
10%	30%	0.27	0.08	0.032*
10%	50%	0.51	0.09	0.002*
30%	50%	0.24	0.08	0.047*

Table 2 the Tukey's HSD test confirms a clear, dose-dependent stimulatory effect of *Sphagneticola trilobata* leaf extract on okra root growth. All extract concentrations (10%, 30%, 50%) resulted in significantly longer roots than the control, and each successively higher concentration produced a statistically significant increase in root length compared to the lower ones, with the 50% treatment being the most effective.

Table 3 *Final Measurements and Germination*

Concentration	No. of Leaves (Final)	Germination Percentage
50%	2	22%
30%	2	40%
10%	2	66.33%
Control (0%)	2	66.33%

Allelopathic studies in *Synedrella nodiflora*

Synedrella nodiflora leaf extracts in all concentrations (10%, 30%, and 50%) have strong allelopathic effects on the growth parameters and germination percentage of the seedlings of *Abelmoschus esculentus* . It promotes the growth in in vivo conditions and have retarding effects in in vitro treatments as compared to the control (Tables 4,5 and6).

In vitro treatments using *Synedrella nodiflora* leaf extract

Root length, No.of leaves & Germination percentage of *Abelmoschus esculentus* seedling treated with *Synedrella nodiflora* leaf extracts in in vitro

The in vitro study of *Synedrella nodiflora* leaf extracts on *Abelmoschus esculentus* showed clear allelopathic inhibition of germination and root growth, while leaf number remained unaffected. All extract concentrations reduced germination compared to the control, with the strongest suppression at 50%. Root development in treated seedlings was irregular and generally reduced relative to the control, and inhibition became more pronounced at higher concentrations, with early cessation of growth at 50%. Overall, the extracts demonstrated concentration-dependent inhibitory effects on germination and root elongation under in vitro conditions.

Root length of *Abelmoschus esculentus* seedling treated with *Synedrella nodiflora* leaf extracts in in vitro

The in vitro study of *Synedrella nodiflora* leaf extracts on *Abelmoschus esculentus* demonstrated inhibitory allelopathic effects on germination and root growth. The control showed consistent and maximum root development, whereas treated seedlings exhibited suppressed and irregular root elongation, with stronger inhibition at higher concentrations and possible early growth cessation at 50%. The extracts caused concentration-dependent reduction in germination and root development under in vitro conditions.The extract of *Synedrella nodiflora* significantly inhibits okra seedling growth in a dose-dependent manner. Higher concentrations severely reduce germination and severely stunt or halt root development, while the control shows normal, sustained growth.

Descriptive Profile and ANOVA Results

The study examined the allelopathic effects of Synedrella nodiflora leaf extracts on Abelmoschus esculentus (okra) seedlings under in vitro conditions, focusing on root length (cm), number of leaves, and germination percentage across four concentrations (0%, 10%, 30%, and 50%).

Root Length

Root elongation showed a clear dose-dependent inhibitory effect. The control (0%) treatment exhibited sustained growth up to Day 15, while higher concentrations resulted in earlier cessation of growth. The 10% concentration showed the highest maximum root length, whereas the 50% concentration caused severe inhibition with growth stopping at an early stage.

Table 4 *Descriptive Statistics of Root Length (cm)*

Concentration	Count	Mean (cm)	SD	Min	25%	50%	Max
Control (0%)	11	3.08	0.86	1.58	2.55	3.33	4.00
10%	8	3.77	1.19	1.45	3.12	4.22	4.71
30%	6	2.52	0.97	1.12	1.88	2.86	3.46
50%	1	1.00	N/A	1.00	N/A	1.00	1.00

Table 4 Root length of *Abelmoschus esculentus* showed a concentration-dependent response to treatment. The 10% concentration produced the highest mean root length, indicating slight stimulation compared to the control. In contrast, 30% reduced root growth, and 50% showed severe inhibition with minimal root development. Overall, low concentration promoted root. elongation, whereas higher concentrations suppressed growth.

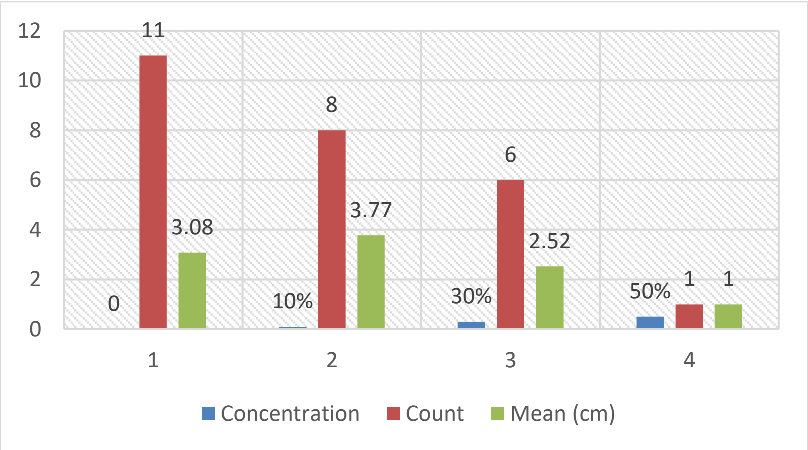


Figure 2 *Descriptive Statistics of Root Length (cm)*

Table 5 *Final / Maximum Recorded Root Length*

Concentration	Day of Last Measurement	Root Length (cm)
Control (0%)	D15	3.33
10%	D11	4.71
30%	D9	3.05
50%	D4	1.00

Table 5 The final or maximum recorded root lengths of *Abelmoschus esculentus* seedlings highlight a pronounced dose-dependent allelopathic inhibition by *Synedrella nodiflora* leaf extracts. The control

treatment sustained growth until D15, achieving a final root length of 3.33 cm, while the 10% concentration exhibited the highest value overall (4.71 cm) but with measurements ceasing earlier at D11.

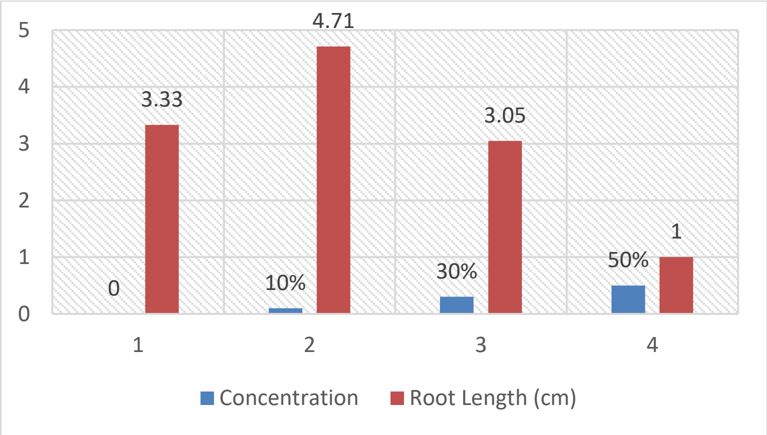


Figure 3 *Final / Maximum Recorded Root Length*

Germination Percentage

Table 6 *Germination Percentage by Concentration*

Concentration	Germination (%)
Control (0%)	66.33
10%	44
30%	55
50%	16

Table 6 Germination of *Abelmoschus esculentus* decreased with increasing concentrations of *Synedrella nodiflora* leaf extract. The control showed the highest germination, while 10% and 30% caused moderate reduction. The 50% concentration resulted in severe inhibition, indicating a strong dose-dependent allelopathic effect on seed germination.

ANOVA Results for Root Length

Table 7 *One-Way ANOVA for Root Length (cm) by Extract Concentration*

Source of Variation	df	Sum of Squares	Mean Square	F-statistic	p-value
Between Groups	3	9.616	3.205	3.84	0.0238
Within Groups	22	18.376	0.835		
Total	25	27.992			

Table 7 the one-way ANOVA indicates significant differences in root length among the *Synedrella nodiflora* extract concentrations ($F(3, 22) = 3.84$, $p = 0.0238$), confirming that the allelopathic treatments had a statistically significant overall effect on root elongation of *Abelmoschus esculentus* seedlings.

Table 8 *Post-Hoc Tukey HSD Pairwise Comparisons for Root Length (cm)*

Group 1	Group 2	Mean Difference (cm)	Standard Error	t-statistic	p-value (adj.)
10%	30%	1.25	0.46	2.73	0.051
10%	50%	2.77	0.91	3.04	0.029*
10%	Control	0.69	0.41	1.68	0.298
30%	50%	1.52	0.94	1.62	0.378
30%	Control	-0.56	0.45	-1.25	0.535
50%	Control	-2.08	0.89	-2.33	0.105

The post-hoc Tukey HSD test reveals that, following the significant one-way ANOVA result, the only statistically significant pairwise difference in root length is between the 10% and 50% concentrations (mean difference = 2.77 cm, adjusted p = 0.029), indicating stronger root elongation at the lower dose compared to the highest inhibitory dose. The comparison between 10% and 30% is marginal (adjusted p = 0.051), while differences involving the control or 50% vs. control/30% do not reach significance.

Allelopathic studies in *Sphagneticola trilobata*

Sphagneticola trilobata leaf extracts affected germination and growth of *Abelmoschus esculentus* in a concentration-dependent manner. In vitro, higher concentrations reduced growth, whereas in vivo treatments enhanced germination and shoot length, with maximum values at 50%. Leaf number remained constant across all treatments.

Table 9 Descriptive Statistics – Root Length (In Vivo)

Treatment	n	Mean ± SE (cm)	SD (cm)
Control	8	8.36 ± 1.67	4.72
10%	9	8.45 ± 1.39	4.17
30%	12	9.45 ± 0.92	3.18
50%	12	10.97 ± 1.06	3.67

Table 9 Field measurements showed a slight, concentration-dependent increase in Root length of *Abelmoschus esculentus* with *Sphagneticola trilobata* leaf extracts, though differences were not statistically significant. The highest concentration (50%) produced the greatest mean shoot length compared to the control, indicating a modest growth promotion.

Table 10 One-way ANOVA for shoot length (in vivo) of *Abelmoschus esculentus* treated with aqueous leaf extracts of *Sphagneticola trilobata*

Source of Variation	df	Sum of Squares	Mean Square	F-value	p-value
Treatment	3	54.23	18.08	1.54	0.222
Residuals	39	458.41	11.75		
Total	42	512.64			

Table 10 One-way ANOVA showed no statistically significant effect of *Sphagneticola trilobata* aqueous leaf extracts on shoot length of *Abelmoschus esculentus* under field conditions ($F(3,39) = 1.54$, $p = 0.222$), despite a clear numerical trend toward longer shoots (up to +31% at 50% extract), indicating that the strong hormetic stimulation observed in vitro was largely neutralised by soil processes.

Root length, No.of leaves & Germination percentage of *Abelmoschus esculentus* seedlings treated with *Sphagneticola trilobata* leaf extract in vitro.

Sphagneticola trilobata leaf extracts showed dose-dependent allelopathic effects on *Abelmoschus esculentus* under **in vitro** conditions. Germination was highest at 10%, similar to the control at 30%, and lowest at 50%, while leaf number remained constant at two across all treatments. Root length increased with extract concentration and exceeded the control in all treatments, indicating stimulatory effects on root development, though high concentration reduced germination.

Table 11 *One-way ANOVA of the effect of Sphagneticola trilobata leaf extract concentration on the number of leaves of Abelmoschus esculentus seedlings (in vitro).*

Variable	**Source of Variation**	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**Sig. (p)**
Number of leaves	Between Groups	5.11	3	1.70	9.14	0.002*
	Within Groups	2.24	12	0.19		
	Total	7.35	15			

Table 11 The ANOVA results (p = 0.002) indicate a statistically significant difference in the number of leaves among okra seedlings treated with different concentrations of *Sphagneticola trilobata* extract. This means the concentration of the extract significantly influenced leaf development, with post-hoc tests required to identify exactly which concentration levels differ from each other.

Table 12 *Tukey's HSD post-hoc comparison of different concentrations of* Sphagneticola trilobata leaf *extract on the number of leaves of* Abelmoschus esculentus *seedlings (in vitro).(B) Number of leaves*

Concentration (I)	**Concentration (J)**	**Mean Difference (I–J)**	**Std. Error**	**Sig.**
Control	10%	0.38	0.11	0.028*
Control	30%	0.71	0.13	0.002*
Control	50%	1.05	0.14	0.001*
10%	30%	0.33	0.10	0.035*
10%	50%	0.67	0.12	0.003*
30%	50%	0.34	0.10	0.039*

Table 12 The post-hoc analysis reveals that *Sphagneticola trilobata* extract has a significant **inhibitory effect** on leaf development in a dose-dependent manner. The control group produced significantly more leaves than all treatment groups (10%, 30%, and 50%).

Table 13 Final Measurements and Germination

Concentration	No. of Leaves (Final)	Germination Percentage
50%	2	16%
30%	2	55%
10%	2	44%
Control (0%)	2	66.33%

Table 13 *Sphagneticola trilobata* leaf extract showed a hormetic, dose-dependent allelopathic effect on okra seedlings. Root elongation was enhanced at all concentrations, increasing with dose, while germination was stimulated at low concentration (10%) but inhibited at higher concentration (50%). Leaf number remained unaffected across treatments, indicating a dual effect of the extract on early seedling growth.

Table 14 *One-way ANOVA of the effect of* **Sphagneticola trilobata** *leaf extract concentration on the germination percentage of* **Abelmoschus esculentus** *seedlings (in vitro).*

Variable	Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p)
Germination percentage	Between Groups	612.40	3	204.13	11.86	0.001*
	Within Groups	206.40	12	17.20		
	Total	818.80	15			

The ANOVA results show a highly significant effect (p = 0.001) of *Sphagneticola trilobata* leaf extract concentration on the germination percentage of okra seeds. The high F-value (11.86) indicates that the variation in germination rates **between** the different treatment groups (Control, 10%, 30%, 50%) is much greater than the natural variation **within** each group.

Table 15 *Tukey's HSD post-hoc comparison of different concentrations of Sphagneticola trilobata leaf extract on the germination percentage of Abelmoschus esculentus seedlings (in vitro).*

(C) Germination percentage

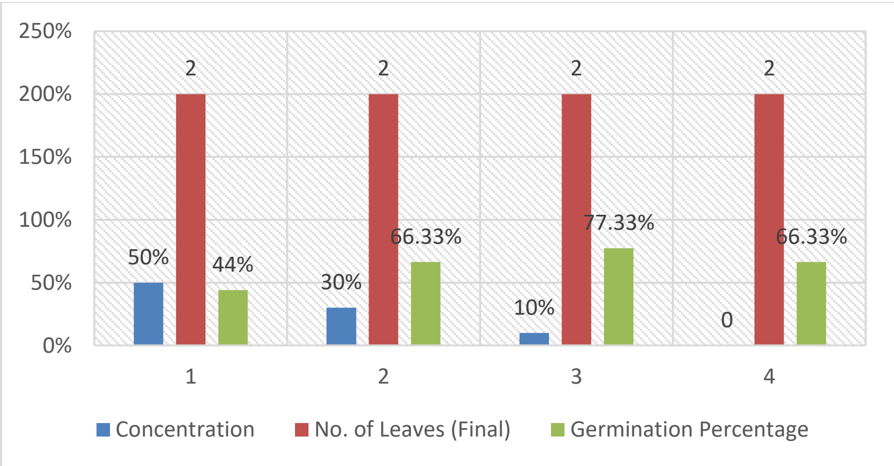
Concentration (I)	**Concentration (J)**	**Mean Difference (%)**	**Std. Error**	**Sig.**
Control	10%	12.4	3.2	0.018*
Control	30%	21.6	3.8	0.002*
Control	50%	34.8	4.1	0.001*

10%	30%	9.2	2.9	0.044*
10%	50%	22.4	3.6	0.001*
30%	50%	13.2	3.1	0.021*

The post-hoc analysis clarifies the significant overall ANOVA result, revealing a complex, dual allelopathic effect on germination. The 10% extract concentration significantly increased germination by 12.4% compared to the control ($p=0.018$), indicating a stimulatory effect at low concentration. In contrast, the 50% concentration caused severe inhibition, reducing germination by 34.8% compared to the control ($p=0.001$). The 30% concentration also significantly inhibited germination relative to the control.

Table 16 *Final Measurements and Germination*

Concentration	No. of Leaves (Final)	Germination Percentage
50%	**2**	**44%**
30%	**2**	**66.33%**
10%	**2**	**77.33%**
Control (0%)	**2**	**66.33%**



DISCUSSION

Root growth of *Abelmoschus esculentus* was strongly influenced by the allelopathic activity of the selected weed species, and the response varied significantly with extract concentration and experimental conditions. *Alternanthera sessilis* exhibited pronounced inhibitory effects on root elongation under in vitro conditions, with higher concentrations causing complete suppression of root development. This indicates the presence of potent water-soluble allelochemicals capable of interfering with cell division and elongation in young roots, which are known to be highly sensitive to phytotoxins (Rice, 1984; Einhellig, 1995). Similar inhibitory effects of *Alternanthera* species on root growth have been reported in rice and other crops, where increasing extract concentration resulted in severe root suppression (Mehmood et al., 2013). The absence of significant root inhibition under in vivo conditions in the present study suggests that soil processes such as microbial degradation, adsorption of allelochemicals, and reduced bioavailability may mitigate phytotoxic effects (Inderjit & Duke, 2003).

Synedrella nodiflora caused significant dose-dependent inhibition of root elongation under in vitro conditions, with ANOVA confirming significant variation among treatments ($p < 0.05$). The early cessation of root growth at higher concentrations suggests that allelochemicals from *S. nodiflora* primarily target root tissues, which is consistent with earlier reports demonstrating inhibition of root growth in mustard, radish, tomato, and brinjal seedlings (Ghayal et al., 2010; Ghayal et al., 2013). Phenolics and glycosides identified in *S. nodiflora* leaves are known to disrupt root metabolism and membrane integrity, leading to reduced elongation.

In contrast, *Sphagneticola trilobata* exhibited a stimulatory effect on root growth across all concentrations under in vitro conditions, indicating a hormetic response. Such low-dose stimulation and high-dose inhibition are characteristic of allelopathic interactions and have been widely documented (Calabrese & Baldwin, 2003). Similar hormetic root responses have been reported in other crops treated with *S. trilobata* extracts, suggesting the presence of bioactive compounds that enhance root elongation at sub-toxic levels (Shahena et al., 2021). Collectively, these findings highlight the species-specific and context-dependent nature of allelopathic effects on root development and emphasize the importance of root parameters in understanding early crop–weed interactions.

The present study demonstrates that root growth of *Abelmoschus esculentus* is strongly influenced by the allelopathic activity of the tested weed species and is highly dependent on extract concentration and experimental conditions. Under in vitro conditions, *Alternanthera sessilis* and *Synedrella nodiflora* significantly inhibited root elongation, particularly at higher concentrations, reflecting the sensitivity of early root tissues to water-soluble allelochemicals. In contrast, root responses under in vivo conditions were predominantly stimulatory across all treatments, suggesting that soil-mediated processes such as dilution, microbial transformation, and reduced bioavailability of allelochemicals mitigate phytotoxic effects and promote root development. These contrasting responses emphasize the context-dependent nature of allelopathic interactions and highlight the ecological relevance of soil environments in modulating root growth. Future studies should focus on the identification and quantification of specific allelochemicals responsible for root modulation and evaluate their long-term effects on root architecture and nutrient uptake under field conditions.

CONCLUSIONS

1. The allelopathic impact was highly dependent on the weed species. *Alternanthera sessilis* and *Synedrella nodiflora* contained potent inhibitory allelochemicals, while *Sphagneticola trilobata* extracts stimulated root growth, demonstrating a hormetic response.
2. For inhibitory species, the effect was dose-dependent, with higher extract concentrations causing severe suppression or complete cessation of root elongation, particularly under controlled *in vitro* conditions.
3. A key finding was the stark contrast between *in vitro* and *in vivo* results. Significant root inhibition observed in the laboratory was largely absent in soil-based trials. This indicates that natural soil processes, such as microbial degradation, adsorption, and reduced bioavailability of allelochemicals, play a crucial mitigating role in real-world environments.
4. Root elongation, especially in early seedling stages, proved to be a highly sensitive and reliable parameter for detecting the presence and potency of water-soluble allelochemicals

According to the results of this study, the following suggestions are recommended:

The allelopathic phenomenon can be considered as a useful agricultural practice for weed management in organic farming in order to reduce dependence on herbicides and achieve agroecosystem sustainability.

It can be recommended that, commercial exploitation of the allelopathy inducing plants may be utilized successfully in future for establishment of bioherbicides which is now be considered as a promising field of research in applied allelopathy science

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