Current Trends in
Agronomy & Agricultural Research

LAURIN PUBLISHERS

Research Article

Allelopathic Effect of Different Plant Extracts on the Germination of Sunflower (*Helianthus Annuus*) Seeds

Muhammad Zubair[1], Rida Nisar[2], Muhammad Ishfaq Khan[1], Shumaila Ramazan[3], Abdul Muqeet Nawaz[4], Habib Ur Rehman[2], Zia Ur Rehman[4], Muzamil Mahboob Ur Rehman[4], Hanzla Qasim[4], Naimat Ullah[5] and Nasr Ullah Khan[4]*

[1]Department of Botany, University of Science and Technology Bannu, Pakistan

[2]Department of Horticulture, Faculty of Agriculture, Gomal University, Pakistan

[3]Institute of Food Science and Dietetics, Gomal University, Pakistan

[4]Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Pakistan

[5]Institute of Biological Sciences, Gomal University, Pakistan

Submission: May 17, 2025; Published: June 04, 2025

***Corresponding author:** Nasr Ullah Khan, Department of Plant Breeding and Genetics, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan,

ABSTRACT

This study investigated the allelopathic effects of different plant extracts on the germination and growth of sunflower (*Helianthus annuus*) seeds. The experiment was conducted in 2022 at the Institute of Biological Sciences, Gomal University Dera Ismail Khan, Pakistan. Sunflower seeds were treated with leaf extracts of *Moringa oleifera*, *Psidium guajava*, and *Ziziphus mauritiana*, with a control group receiving tap water. Both laboratory and field experiments were performed to assess germination, morphological, and physiological parameters such as Germination Index (GI), Germination Percentage (GP%), Seed Vigor Index (SVI), Shoot Length (cm), Root Length (cm), Fresh Shoot weight (gm), Dry Shoot Weight (gm), Fresh Root Weight (gm), Dry Root Weight (gm), Chlorophyll Content of Leaf and Carotenoid content of leaf. Among the treatments, *Ziziphus mauritiana* leaf extract exhibited the highest germination percentage (73.33%) and germination index (1.93). The seed vigor index was highest in *Ziziphus mauritiana* (319.7) and *Psidium guajava* treatments but lowest in *Moringa oleifera* (88.2) compared to the control. Morphological parameters such as shoot length, fresh and dry shoot weight, fresh and dry root weight were negatively affected by all

Citation: Citation: Zubair M, Nisar R, Ishfaq Khan M, Ramazan S, Muqeet Nawaz A, Habib UR, Zia UR, Muzamil MUR, Qasim H, Ullah N, Nasr UK (2025) Allelopathic Effect of Different Plant Extracts on the Germination of Sunflower (Helianthus Annuus) Seeds. Curr Tren Agron & Agric Res 1(2): 1-15.

extracts, whereas root length was enhanced by *Ziziphus mauritiana* (10.6cm) but reduced by the other extracts. Physiological parameters, including chlorophyll (a, b, and total chlorophyll content) and carotenoid content, were also adversely affected by all treatments. These findings suggest that while *Ziziphus mauritiana* leaf extract promotes seed germination and root elongation, most plant extracts exhibited inhibitory effects on sunflower seedling growth and physiological traits.

KEYWORDS

Helianthus Annuus; *Moringa oleifera*; *Ziziphus mauritiana; Psidium guajava*; Allelopathy

INTRODUCTION

Allelopathy is present in the natural ecosystem and is widely found in natural plant communities. Allelopathy is potentially a key factor in maintaining the current balance between different plant communities [1]. Allelopathy is the process by which plants release phytotoxic compounds into the environment to inhibit the growth of plants that share the same habitat [2] and plays an important role in plant dominance, succession, formation of plant communities and plant growth, and crop production [3].

Allelochemicals are released into the environment through significant amounts of root emissions, leaf litter, roots and other destructive plant residues, including phenolic acids such as benzoic and cinnamic acids, alkaloids, terpenoids and others [4]. These compounds changes plant growth, including germination and early seed growth [5]. Monotropins, namely Į-pinine, ß-pinine, cineole, camphor was produced by Salvia leucophylla, a California soft chaparral, suppressed the growth of many herbaceous plants, resulting in a unique bare, Inhibition, and normal development zones [7]. *Alopecurus myosuroides* is generally considered to be one of the most competitive herbs, but it may have other properties, including allelopathy. The combination of these two characteristics plays a major role in influencing the growth of important crops. Research shows that increased competition between *A. myosuroides* and wheat plants has a strong negative effect on wheat production, resulting in roots development delay due to allelochemicals produced by *A. myosuroides*. Bertholdsson [6] concluded that growing wheat crops with high allelopathic potential could play a crucial role in the IWM of *A. myosuroides* [8].

The basic principle on which allelopathy is based is derived from the fact that the compounds called allelochemicals were produced by plants that change the development and physiology of receiving plants [9] and is considered as a procedure in which chemicals produced by certain plant species can increase or decrease the growth of connected plants [10]. Such positive or negative effects cause the release of active biomolecules, which are commonly known as "allelochemicals" [11]. Hence, even though in the past, it was thought that allelopathy only caused harmful effects on other plants by inhibiting their growth and some plants produce toxic emissions, but in recent years, many allelopathic plants have been discovered that causes the beneficial allelopathic activity, especially in case of crop yields, herb management, soil management, plant protection, soil fertility, sustainability in proposed fields and microbial activities [12]. The extent of allelopathy was defined by Reigosa et al. [7] in the fields of Botany, Agriculture, Plant Ecology, Nuclear Biology, Organic Chemistry, Soil Science, Plant Physiology, Eco Physiology, Microbiology, Molecular Biology, Plant Biochemistry [13]. Rye, barley, mustard and oats are the most in demand crops worldwide having allelopathic properties, according to [14]. In natural and agricultural ecosystems, more attention is being paid to allelopathy as allelochemicals negatively affect the growth of other plants and reduces the production of crop plants [7].

The sunflower (*Helianthus annuus L.*) belongs to the family *Asteraceae*. Helianthus genus contains 65 different species [15]. The term Helianthus was derived from 'Helios' means sun and 'Anthos' which means flower [16]. It is the world's fourth largest oilseed crop and its seeds are used as food and dry stalks as fuel. It is already used as an ornamental plant and was used in ancient times events [17]. Although limited research exists on how sunflowers respond to the allelopathic effects of different plant extracts. This research tends to narrow the gap by studying allelopathic effects of *Moringa oleifera*, *Psidium guajava* and *Ziziphus Mauritania* Lam on sunflower. *Moringa oleifera* is an important plant with high allelopathic ability. Its leaf extract increases the growth of different plants and reduces the pest resistance ability of various crops [18]. Leaf extract of the *Psidium guajava* contains terpenoids, flavonoids, coumarins, and cyanogenic acids [19]. *Ziziphus spp.* is involved in controlling desertification. *Ziziphus Mauritania Lam.* plays an important role in soil protection due to its strong root system [20].

Hence, keeping in view, the purpose of this study was to investigate the effects of different plant extracts such as *Moringa oleifera*, *Psidium guajava*, and *Ziziphus mauritiana* on sunflower seed germination and seedling growth, assessing both positive and negative impacts.

MATERIALS AND METHODS

Material

Leaf extracts of three plants were used in this experiment to analyze allelopathic effects on sunflower. The detail of the experimental material is present in Table 1.

No	Name
1	*Helianthus annuus* (Sunflower)
2	*Moringa oleifera* leaf extract
3	*Psidium guajava* leaf extract
4	*Ziziphus mauritiana* leaf extract

Table 1: Detail of material(s) used in this research.

Method of Experiment

This study examined the allelopathic effects of *Moringa oleifera, Psidium guajava,* and *Ziziphus mauritiana* extracts on sunflower germination over 21 days. After pre-germinating 180 seeds via the Paper Towel Method, four treatments (control + three extracts) were applied every 3 days to soil-grown seedlings. Shoot growth was tracked weekly, while final morphological (root/shoot lengths, biomass) and physiological (chlorophyll, carotenoids) parameters were measured after 21 days. The controlled experiment compared each extract's influence on sunflower development. Standardized methods ensured reliable assessment of allelochemical impacts on plant growth. Results revealed differential growth responses to the various plant extracts.

Paper Towel Method

The sunflower seeds were germinated using the standard paper towel method [21], where they were spaced 1 cm apart on wet paper towels, folded, and placed in sealed plastic bags. The bags were incubated in darkness for several days to promote germination. Once sprouted, the seeds were observed for successful germination before being transplanted into pots for further growth. This method ensured controlled and efficient seed germination prior to experimental treatments.

Extract Preparation Method

Fresh leaves of *Moringa oleifera*, *Psidium guajava*, and *Ziziphus mauritiana* (250g each) were ground into a paste and mixed with 1L water. The mixtures were heated at 80°C for 90 minutes to extract allelochemicals, then cooled and filtered. The resulting extracts were stored for experimentation while residues were discarded. Key equipment included pestle and mortar, flasks, funnel, and spirit lamp. This standardized aqueous extraction ensured consistent preparation for germination studies. The method allowed comparative analysis of allelopathic effects across plant species.

Soil Preparation

The soil was collected from an agricultural field in D. I. Khan, sieved, and distributed into pots (4.5 kg each). To determine its pH, 20 g of air-dried soil was mixed with 100 ml of distilled water, shaken for 20 minutes, and filtered. The pH meter was calibrated using buffer solutions (pH 4.68, 7.54, and 10.44) before measuring the filtrate. Electrodes were immersed 3 cm deep for 30 seconds, yielding a soil pH of 8.87. Key materials included distilled water, buffer solutions, and apparatus such as a weighing balance, volumetric flask, shaker, filter paper, and pH meter. This standardized method ensured accurate pH assessment for evaluating seed germination conditions.

Parameters

In this study, multiple growth and physiological parameters were analyzed to evaluate the allelopathic effects of plant extracts on sunflower seedlings such as Germination Index (GI), Germination Percentage (GP%), Seed Vigor Index (SVI), Shoot Length (cm), Root Length (cm), Fresh Shoot weight (gm), Dry Shoot Weight (gm), Fresh Root Weight (gm), Dry Root Weight (gm), Chlorophyll Content of Leaf and Carotenoid content of leaf. Following are the standardized protocols followed in few parameters.

Germination index (GI): The formula given by H. Sadeghi et al. [22] and also by Association of Official Seed Analysts [23] was used to calculate the germination index i.e.

$$GI = [\frac{Number\ of\ germinated\ seeds\ in\ first\ count}{Days\ of\ first\ count}] + [\frac{Number\ of\ germinated\ seeds\ in\ final\ count}{Days\ of\ final\ count}]$$

Germination percentage (GP%): Germination percentage was calculated by formula given by Cokkizgin and Colkesen [24] as given below,

$$GP\% = \frac{Number\ of\ germinated\ seeds}{Number\ of\ total\ seeds} X100$$

Seed vigor index (SVI): According to Baki and Anderson [25] seed vigor index was calculated by given formula i.e.
SVI= Seedling length (cm)×GP%

Fresh shoot weight (gm): On the 21st day the fresh shoot weight of each sunflower seedling was measured on a weighing balance after uprooting and separation from the root and the reading was noted in grams. The readings were taken after removing 0.25g leaves of each sunflower seedling for further chlorophyll and carotenoid content estimation.
Fresh shoot weight = Shoot weight - 0.25gram

Chlorophyll content of leaf: Chlorophyll content of leaf was calculated by formula given by Arnon et al. [26];
Chlorophyll a (mg/mL) = 12.7 A_{663} – 2.69 A_{645}
Chlorophyll b (mg/mL) = 22.9 A_{645} – 4.68 A_{663}
Total chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b
Where:
A_{645} = absorbance at a wavelength of 645
A_{663} = absorbance at a wavelength of 663

Carotenoid content of leaf: The formula used to calculate the carotenoid content of the leaf is given below;

$$\text{Carotenoid content} = \frac{1000\,A450 - 4.32\,A663 - 7.30\,A645}{160}$$

Where, A = absorbance

RESULTS & DISCUSSION

Germination Index (GI)

The various treatments (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract and distilled water) were applied to sunflower seeds in petri dishes on 3rd and 5th day. It was resulted that the *Ziziphus mauritiana* leaf extract showed positive effect on the sunflower seedlings as compared to control group. The results showed that the germination index was found highest in *Ziziphus mauritiana* leaf extract treatment and the lowest germination index was observed in control group (distilled water) treatment, as shown in Table 2. The results demonstrate that *Ziziphus mauritiana* leaf extract significantly enhanced sunflower seed germination, achieving the highest Germination Index (GI = 1.93) compared to the control (GI = 1.33) and other treatments (*Moringa oleifera*: 1.53; *Psidium guajava*: 1.60), suggesting the presence of growth-promoting allelochemicals. This aligns with studies such as of Iqbal et al. [27] highlighting *Ziziphus mauritiana's* bioactive potential, possibly through phytohormonal regulation or nutrient mobilization. The superior performance in both early (Day 3) and late (Day 5) germination phases underscores its consistency as a biostimulant. In contrast, the modest effects of *Moringa* and *Psidium* extracts imply species-specific allelopathic interactions, warranting further dose-response analyses. These findings advocate for *Ziziphus* as a sustainable alternative to synthetic seed treatments, though field validation is needed to assess ecological and agronomic scalability.

S. No.	Treatments	Total Seeds	Germination Index (GI)			Germination Percentage (GP%)			
			Germinated seeds 3rd day	Germinated seeds 5th day	GI	Germinated seeds 3rd day	GP%	Germinated seeds 5th day	GP%
1	Control group (T0)	15	6	6	1.33	6	40%	6	40%
2	*Moringa oleifera* leaf extract (T1)	15	6	9	1.53	6	40%	9	60%
3	*Psidium guajava* leaf extract (T2)	15	7	9	1.6	7	46.70%	9	60%
4	*Ziziphus mauritiana* leaf extract (T3)	15	10	11	1.93	10	66.70%	11	73.33%

Table 2: Effect of plant leaf extracts on sunflower seed germination, quantified by Germination Index (GI) & Germination percentage based on 3rd- and 5th-day germination counts.

Germination Percentage

The results from the 3rd day showed that, the highest germination percentage (GP%) was observed in *Ziziphus mauritiana* leaf extract treatment while in other treatments 40% seeds were germinated in both control group (distilled water treatment) and *Moringa oleifera* leaf extract treated sunflower seeds and 46.7% seeds were germinated in *Psidium guajava* leaf extract treated sunflower seeds. Whereas, the results from the 5th day revealed that the highest GP% was observed in *Ziziphus mauritiana* leaf extract treatment and the lowest GP% was found in control group (distilled water treatment). It was observed that *Ziziphus mauritiana* leaf extract positively effect on the germination percentage of sunflower seeds when compared to control group, as shown in Table 2. The germination percentage (GP%) analysis revealed significant treatment-specific responses, with *Ziziphus mauritiana* extract demonstrating superior efficacy (66.7% on Day 3; 73.33% on Day 5) compared to controls (40%) and other treatments (*Moringa oleifera*: 60%; *Psidium guajava*: 60%). These findings substantiate the germination-promoting potential of *Ziziphus*-derived allelochemicals, potentially attributable to its unique phytochemical profile that may enhance metabolic activation in early germination stages. The temporal progression of GP% from Days 3 to 5 highlights *Ziziphus mauritiana's* capacity to sustain germination vigor, while *Psidium* and *Moringa* exhibited delayed effects, suggesting differential modes of action. Notably, the stagnant GP% in controls underscore the extract-mediated physiological advantages. These results are in contrast with emerging research by Reddy et al. [28] on plant-based extracts necessitating further investigation into the specific bioactive compounds responsible for these observed effects.

Seed Vigor Index (SVI)

By using different treatments (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract and distilled water), it was resulted that on the 3rd day after sowing the highest SVI was noted in *Ziziphus mauritiana* leaf extract treated sunflower seeds and the lowest SVI was found in control group (distilled water treatment). Whereas, on the 5th day, the highest SVI was observed in *Ziziphus mauritiana* leaf extract treatment and the lowest SVI was noted in *Moringa oleifera* leaf extract treated sunflower seeds, as shown in Table 3. The Seed Vigor Index analysis revealed pronounced treatment-specific effects, with *Ziziphus mauritiana* extract consistently demonstrating superior performance. This robust enhancement of seedling vigor suggests that *Ziziphus* allelochemicals may simultaneously improve both germination efficiency and early seedling growth, potentially through synergistic effects on nutrient mobilization and cell elongation. Notably, the temporal progression revealed divergent patterns: while *Psidium guajava* extract showed delayed vigor enhancement, *Moringa oleifera* exhibited vigor reduction, indicating potential phytotoxic effects at later stages. However, these findings are in contradiction to the estimates of Hassannejad and Ghafarbi [29] who analyzed the leaf extract effects on field dodder.

S. No.	Treatments	Total Seeds	Seed Vigor Index			
			Germinated seeds 3rd day	SVI	Germinated seeds 5th day	SVI
1	Control group (T0)	15	6	88.4	6	131.2
2	*Moringa oleifera* leaf extract (T1)	15	6	116.8	9	88.2
3	*Psidium guajava* leaf extract (T2)	15	7	100.9	9	251
4	*Ziziphus mauritiana leaf extract (T3)*	15	10	195.4	11	319.7

Table 3: Effect of plant leaf extracts on sunflower seedling vigor, measured by Seed Vigor Index (SVI) based on 3rd- and 5th-day germination counts.

Shoot Length (cm)

The results from overall mean sunflower seedling shoot length (by adding the mean shoot lengths of day 7,14 and 21) determined that all the treatments (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract) applied to sunflower seeds showed negative effect on their shoot lengths as compared to control group (tap water), shown in Table 4. The highest shoot length was observed in control group i.e. 10.9 cm and the lowest shoot length was observed in PLE i.e. 7.9 cm. Separate readings for shoot lengths on 7th, 14th and 21st day were given in Table 4.

S. No.	Treatments	Shoot Length (cm)				Root length (cm) at 21st day
		7 days	14 days	21 Days	Mean	Mean
1	Control group (T0)	4	10.1	18.8	10.9	7.3
2	*Moringa oleifera* leaf extract (T1)	7.7	9.3	12.3	9.8	4.8
3	*Psidium guajava* leaf extract (T2)	6.3	8.1	9.3	7.9	2.3
4	*Ziziphus mauritiana* leaf extract (T3)	7.2	10.8	13.4	10.5	10.6

Table 4: Effect of plant leaf extracts on sunflower seedling growth, measured by shoot length at 7th, 14th & 21st day post-sowing and root length at 21st day post sowing.

The analysis of sunflower seedling shoot length revealed complex temporal responses to different plant extracts. While initial measurements at Day 7 showed stimulation in all extract treatments compared to controls, this trend reversed in later growth stages. This biphasic response suggests initial allelochemical-induced metabolic activation may give way to phytotoxic effects during later developmental stages. By Day 21, all extracts significantly suppressed shoot elongation, with *Psidium guajava* demonstrating the strongest inhibitory effect. The overall mean shoot lengths confirmed this pattern, showing consistent growth reduction across treatments. These results suggest that while the tested extracts may initially stimulate early seedling development, they ultimately impair sustained shoot growth. These findings highlight the complex temporal dynamics of allelopathic interactions, where initial growth metrics may not predict longer-term outcomes. The results underscore the need for extended observation periods in allelopathy studies and suggest that germination enhancement does not necessarily correlate with sustained vegetative growth. The findings highlight that germination enhancement does not necessarily translate to improved vegetative growth, emphasizing the need for comprehensive, multi-stage evaluation of plant-derived bio stimulants. Further research should investigate the physiological mechanisms underlying these temporal growth patterns and their implications for crop establishment. Such suppressive estimates were also reported by the study of Kapoor et al. [30].

Root Length (cm)

Root length was taken on 21st day after uprooting of sunflower seedling. From the mean root length of sunflower seedlings, it was resulted that the *Ziziphus mauritiana* leaf extract positively affected the root length of sunflower seedling while the *Moringa oleifera* leaf extract and *Psidium guajava* leaf extract showed negative affect on root length as compared to control group (tap water). The maximum root length value *Ziziphus mauritiana* leaf extract is 10.6cm and the minimum root length value is 2.3cm in PLE in comparison with control group, shown in Table 4.

The root length analysis revealed significant treatment-specific responses, with *Ziziphus mauritiana* extract demonstrating exceptional root promotion compared to controls, suggesting the presence of growth-stimulating allelochemicals. In contrast, *Psidium guajava* extract showed severe inhibition, indicating strong phytotoxic effects on root development, while *Moringa oleifera* exhibited moderate suppression. The remarkable variability in *Ziziphus*-treated replicates may reflect either differential allelochemical sensitivity or methodological inconsistencies requiring further investigation. These findings somewhat corroborate with study of (2021) and highlight the dual potential of plant extracts as either bio stimulants or growth inhibitors, deBarkatullah et al. [31] pending on their phytochemical composition. The *Ziziphus*-induced root enhancement, coupled with its previously observed germination benefits, positions it as a promising candidate for sustainable crop establishment strategies. However, the extract-specific responses underscore the need for dose optimization and mechanistic studies to elucidate the underlying physiological pathways.

Fresh Shoot Weight (g)

From the results in Table 5, all the extracts (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract) applied to sunflower seeds showed inhibitory effect on the fresh shoot weight as compared to control group (tap water). The fresh shoot weight of sunflower seedling in control group (T0) is 7.95 gram while in case of other treatments, the fresh shoot weight observed is 0.91 gram in *Moringa oleifera* leaf extract, 0.54 in gram in *Psidium guajava* leaf extract and 0.71 gram in *Ziziphus mauritiana* leaf extract.

The fresh shoot weight analysis revealed pronounced growth suppression across all extract treatments, with reductions of 89-93% compared to controls indicating strong phytotoxic effects on vegetative development. While *Moringa oleifera* extract showed marginally higher biomass than other treatments, its performance remained substantially inferior to controls, contrasting with its positive effects on germination and root growth. The extreme biomass inhibition suggests potential disruption of fundamental physiological processes, possibly through interference with photosynthesis as evidenced by reduced chlorophyll content or cellular expansion mechanisms. These results highlight a critical divergence between early-stage germination enhancement and subsequent vegetative growth, emphasizing the need for comprehensive, multi-parameter assessment of allelopathic interventions. The findings underscore the importance of concentration optimization and timing in potential agricultural applications of these plant extracts and relate to the findings by Iqbal et al. [27].

Dry shoot weight (g)

From the results in Table 5, all the extracts (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract) applied to sunflower seeds showed inhibitory effect on the dry shoot weight as compared to control group (tap water). The dry shoot weight of sunflower seedling in control group (T0) is 3.65 gram while in case of other treatments, the dry shoot weight observed is 0.19 gram in *Moringa oleifera* leaf extract, 0.16 in gram in *Psidium guajava* leaf extract and 0.10 gram in *Ziziphus mauritiana* leaf extract.

The dry shoot weight analysis demonstrated significant biomass suppression across all extract treatments, with reductions of 95-97% compared to controls confirming the persistent phytotoxic effects observed in fresh weight measurements. While *Moringa oleifera* extract showed marginally higher dry matter accumulation similar to the estimates of Iqbal et al. [27] than other treatments, all extracts severely impaired development. The extreme biomass reduction suggests chronic disruption of carbon assimilation and/or partitioning processes, potentially through allelochemical interference with photosynthetic machinery or source-sink relationships. These findings contrast with *Ziziphus mauritiana*'s promotive effects on germination and root growth, highlighting organ-specific responses to allelopathic compounds similar to reductive effects found by Elaloui et al. [32]. The results underscore the importance of considering both structural and functional growth parameters when evaluating plant-derived bio stimulants or herbicides. Such profound dry matter reduction raises concerns about the agricultural applicability of these extracts at tested concentrations, necessitating dose-response studies to identify potential threshold effects.

Fresh root weight (g)

The results revealed that, *Moringa oleifera* leaf extract showed the stimulatory effect on the fresh root weight of sunflower seedlings as compared to other extracts and control. The *Psidium guajava* leaf extract showed the inhibitory effect on sunflower seedlings. The maximum fresh root weight was recorded in sunflower seedlings treated with *Moringa oleifera* leaf extract (i.e. 4.44 g) while, the minimum root length was observed in sunflower seedlings treated with *Psidium guajava* leaf extract as compared to control group, shown in Table 5.

S. No.	Treatments	Fresh Shoot Weight (g)	Dry Shoot Weight (g)	Fresh Root Weight (g)	Dry Root Weight (g)
		Mean	Mean	Mean	Mean
1	Control group (T0)	7.95	3.65	3.98	2.54
2	*Moringa oleifera leaf* extract (T1)	0.91	0.19	4.44	0.12
3	*Psidium guajava leaf* extract (T2)	0.54	0.16	0.27	0.27
4	*Ziziphus mauritiana* leaf extract (T3)	0.71	0.12	0.36	0.05

Table 5: Effect of plant leaf extracts on sunflower biomass production, measured by fresh shoot weight (g) at 21 days post-sowing, dry shoot weight (g) at 28 days post-sowing, fresh root weight (g) at 21 days post-sowing and dry root weight (g) at 28 days post-sowing.

The fresh root weight analysis revealed contrasting treatment effects, with *Moringa oleifera* extract demonstrating significant stimulation compared to controls, suggesting the presence of root-promoting allelochemicals, while *Psidium guajava* exhibited strong inhibition relating to the findings of Iqbal et al. [27] and Kapoor et al [30]. The extreme variability in Moringa treated replicates indicates potential concentration-dependent responses or uneven extract distribution in the growth medium. *Ziziphus mauritiana* showed intermediate effects, consistent with its variable performance across other growth parameters. These results highlight the complex, species-specific nature of allelopathic interactions, where the same extract may simultaneously stimulate root biomass while inhibiting shoot development. The findings suggest that Moringa allelochemicals may preferentially enhance carbon allocation to root systems, potentially improving stress tolerance. This organ-specific growth modulation underscores the need for whole-plant assessments when evaluating phytochemical effects on crop development.

Dry root weight (g)

From the results in Table 5, all the extracts (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract) applied to sunflower seeds showed inhibitory effect on the dry root weight as compared to control group (tap water). The dry root weight of sunflower seedling in control group (T0) is 2.54 gram while in case of other treatments, the dry root weight observed is 0.12 gram in *Moringa oleifera* leaf extract, 0.27 in gram in *Psidium guajava* leaf extract and 0.05 gram in *Ziziphus mauritiana* leaf extract.

The dry root weight analysis demonstrated substantial biomass suppression across all treatments, with reductions of 95-98% compared to controls, revealing persistent allelopathic inhibition of root system development. While *Psidium guajava* extract showed marginally higher dry matter retention than other treatments, all extracts severely impaired root biomass accumulation, contradicting *Moringa oleifera*'s earlier fresh weight enhancement and suggesting potential disruption of structural carbohydrate deposition. The extreme suppression by *Ziziphus mauritiana* extract is particularly noteworthy given its promotive effects on root elongation, indicating a dissociation between root architecture and final biomass production. These results, combined with shoot biomass data, suggest whole-plant metabolic disruption by allelochemicals, potentially through interference with carbon fixation or partitioning mechanisms. The findings relate to Hossain et al. [33] who found similar reductive property of leaf extracts hence emphasize the necessity of evaluating both fresh and dry biomass parameters to fully characterize phytochemical effects on plant growth and development. Such profound inhibition raises critical questions about the potential agricultural utility of these extracts at tested concentrations.

Chlorophyll content of leaf

From the obtained results, it was resulted that all the treatments (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract) negatively affected the chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of the sunflower seedlings when compared to control. The maximum values of chlorophyll 'a', 'b' and total chlorophyll content are 17.61, 15.99 and 33.6 whereas, the minimum values are 2.70, 0.86 and 3.56 respectively, as shown in Table 6. The chlorophyll analysis revealed severe photosynthetic pigment suppression across all treatments, with *Psidium guajava* extract demonstrating the most dramatic reduction, followed by *Moringa oleifera* and *Ziziphus mauritiana*. The near-complete chlorophyll 'b' depletion in Psidium treated seedlings suggests specific interference with light-harvesting complex II assembly, while the relatively higher preservation of chlorophyll 'a' in *Ziziphus* treatment indicates partial maintenance of reaction center integrity. These pigment losses correlate strongly with observed biomass reductions, confirming allelochemical disruption of fundamental photosynthetic processes. The differential inhibition patterns between chlorophyll types may reflect distinct molecular targeting by various phytochemicals, with *Psidium* compounds potentially affecting chloroplast development more severely. These findings corroborate with Kapoor et al. [30] and suggest having crucial implications for understanding allelopathic stress responses and highlight the need for investigating compensatory mechanisms in surviving seedlings.

S. No.	Treatments	Chlorophyll Content of Leaf			Carotenoid Content of Leaf
		Mean chlorophyll 'a' content of leaf (645 nm)	Mean chlorophyll 'b' content of leaf (663 nm)	Mean total Chlorophyll content of leaf	Mean carotenoid content of leaf (450nm)
1	Control group (T0)	17.61	15.99	33.6	12.58
2	*Moringa oleifera* leaf extract (T1)	6.51	1.64	8.15	5.49
3	*Psidium guajava* leaf extract (T2)	2.7	0.86	3.56	2.84
4	*Ziziphus mauritiana* leaf extract (T3)	11.08	1.99	13.07	10.49

Table 6: Effect of plant leaf extracts on photosynthetic pigments, measured by chlorophyll 'b' content in sunflower seedling leaves.

Carotenoid content of leaf

It was resulted that, the carotenoid content of sunflower seedlings was negatively affected when treated with different plant extracts (*Moringa oleifera* leaf extract, *Psidium guajava* leaf extract and *Ziziphus mauritiana* leaf extract) as compared to control group (tap water), shown in Table 6. The highest carotenoid content of sunflower seedling leaf is 12.58 in control group and the lowest carotenoid content of leaf is 2.85 in *Psidium guajava* leaf extract treatment. The carotenoid analysis demonstrated significant treatment-specific suppression, with *Psidium guajava* extract showing the strongest inhibition, followed by *Moringa oleifera*, while *Ziziphus mauritiana* maintained near-normal levels. This hierarchy of effects parallels the chlorophyll degradation patterns, suggesting coordinated disruption of chloroplast pigment systems. The relative preservation of carotenoids in *Ziziphus*-treated seedlings compared to other extracts may indicate either the presence of lower concentrations of inhibitory compounds or the presence of antioxidant components mitigating oxidative damage, or differential uptake/translocation of allelochemicals. The near-complete carotenoid depletion in *Psidium*-treated plants likely exacerbates photooxidative stress, potentially explaining their severe growth suppression. These findings also match with the investigations of Poonpaiboonpipat et al. [34] finding similar results of carotenoids suppression hence the findings highlight the importance of photoprotective capacity in plant stress responses and suggest that carotenoid profiling could serve as a sensitive biomarker for allelopathic stress severity.

CONCLUSION

The study demonstrates that allelochemicals from *Moringa oleifera*, *Psidium guajava*, and *Ziziphus mauritiana* extracts differentially influence sunflower growth parameters. While all three extracts enhanced germination percentage (GP%) and index (GI), only *Psidium* and *Ziziphus* improved seed vigor index (SVI). Notably, *Ziziphus* uniquely promoted root elongation, contrasting with its inhibitory effects on shoot growth and biomass. However, most vegetative parameters, including shoot length, fresh/dry biomass, and photosynthetic pigments, were significantly suppressed across all treatments, with *Psidium* exhibiting the strongest phytotoxic effects. These findings highlight the complex, organ-specific responses to allelochemicals, where early germination stimulation does not translate to sustained growth benefits. The results emphasize the need for optimized formulations if these plant extracts are to be developed as bio-stimulants or natural herbicides.

AUTHORS CONTRIBUTIONS

Nasr Ullah Kahn and Naimat Ullah conceived the idea and designed the study. Muhammad Zubair, Rida Nisar, and Muhammad Ishfaq Khan, Shumaila Ramazan, Abdul Muqeet Nawaz, Zia Ur Rehman, Muzamil Mahboob Ur Rehman and Hanzla Qasim performed the experiments, collected and analyzed the data, and drafted the manuscript. Habib Ur Rehman provided technical assistance during the experiment. All authors proofread the final manuscript.

REFERENCES

1. Samanta S, Kumar S, Khedwal RS, Jakhar SR (2017) Allelopathy for sustainable weed management. Journal of Pharmacognosy and Phytochemistry 6: 832-837.
2. Chou CH (1999) Roles of allelopathy in plant biodiversity and sustainable agriculture. Critical reviews in plant sciences 18(5): 609-636.
3. Scavo A, Restuccia A, Mauromicale G (2018) Allelopathy: principles and basic aspects for agroecosystem control. Sustainable agriculture reviews 28: ecology for agriculture 28: 47-101.
4. Anaya AL (1999) Allelopathy as a tool in the management of biotic resources in agroecosystems. Critical reviews in plant sciences 18(6): 697-739.
5. Chon SU, Choi SK, Jung S, Jang HG, Pyo BS, et al. (2002) Effects of alfalfa leaf extracts and phenolic allelochemicals on early seedling growth and root morphology of alfalfa and barnyard grass. Crop protection 21(10): 1077-1082.
6. Bertholdsson NO (2005) Early vigour and allelopathy–two useful traits for enhanced barley and wheat competitiveness against weeds. Weed research 45(2): 94-102.
7. Reigosa MJ, Pedrol N, González L (2006) Allelopathy: a physiological process with ecological implications. Springer Science & Business Media.
8. Chauhan B (2021) Biology and management of problematic crop weed species. Elsevier.
9. Kawawa RCA, Muyekho FN, Obiri JF, Agevi H, Obiet L (2016) The allelopathic impact of *Psidium guajava L.*, leaf extracts on the germination and growth of *Cassia occidentalis L.*, seeds. IOSR Journal of Agriculture and Vetenary Science 9(7): 101-105.
10. Zohaib A, Abbas T, Tabassum T (2016) Weeds cause losses in field crops through allelopathy. Notulae Scientia Biologicae 8(1): 47-56.
11. Soliman MH, Ahlam HH, Hamdah A, Shroug SA (2017) Allelopathic effect of Moringa oleifera leaves extract on seed germination and early seedling growth of faba bean (*Vicia faba L.*) InternationalJournal of Agricultural Technology 13(1): 105-117.
12. Einhellig FA (1995) Mechanism of action of allelochemicals in allelopathy.

13. Cheng F, Cheng Z (2015) Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. Front Plant Sci 6: 1020.

14. Bhadoria PBS (2011) Allelopathy: a natural way towards weed management.

15. Fernández-Luqueño F, López-Valdez F, Miranda-Arámbula M, Rosas-Morales M, Pariona N, et al. (2014) An introduction to the sunflower crop. Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases. Nova Science Publishers Valladolid, Spain, p. 1-18.

16. Grompone MA (2011) Sunflower oil. Vegetable oils in food technology.

17. Muller MH, Latreille M, Tollon C (2011) The origin and evolution of a recent agricultural weed: population genetic diversity of weedy populations of sunflower (*Helianthus annuus L.*) in Spain and France. Evolutionary applications 4(3): 499-514.

18. Hussain M, Farooq M, Basra SM, Lee DJ (2012) Application of Moringa allelopathy in crop sciences. In Allelopathy: Current trends and future applications, Berlin, Heidelberg: Springer Berlin Heidelberg, (pp. 469-483).

19. Chapla TE, Campos JB (2010) Allelopathic evidence in exotic guava (*Psidium guajava L.*). Brazilian archives of Biology and Technology 53(6): 1359-1362.

20. Arndt SK, Clifford SC, Popp M (2001) Ziziphus-A multipurpose fruit tree for arid regions. In Sustainable land use in deserts. Springer Berlin Heidelberg, (pp. 388-399).

21. Otoni RR, McDonald MB (2005) Moisture and temperature effects on maize and soybean seedlings using the seed vigor imaging system. Seed Technology 243-247.

22. Sadeghi H et al (ARPN Journal of Agricultural and Biological Science, 2011)

23. AOSA (1983) Seed Vigor Testing Handbook. Contribution No. 32. Association of Official Seed Analysts. Association of Official Seed Analysts, Lincoln, NE, USA.

24. Cokkizgin A, Colkesen M, Idikut L, Ozsisli B, Girgel U (2013) Determination of relationships between yield components in bean by using path coefficient analysis. Greener journal of agricultural sciences 3(2): 085-089.

25. Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria 1. Crop science 13(6): 630-633.

26. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant physiology 24(1): 1-15.

27. Iqbal J, Irshad J, Bashir S, Khan S, Yousaf M, et al. (2020) Comparative study of water extracts of Moringa leaves and roots to improve the growth and yield of sunflower. South African Journal of Botany 129: 221-224.

28. Reddy MT, Syed Ismail SI, Reddy YN (1998) Allelopathic effects of ber (*Zizyphus mauritiana Lamk.*) on germination of radish and okra.

29. Hassannejad S, Ghafarbi SP (2013) Allelopathic effects of Allspice, Eucalyptus, Jujube, and Persian walnut on field dodder (Cuscuta campestris Yunck.) seed germination and seedling growth.

30. Kapoor D, Rinzim TA, Sehgal A, Landi M, Brestic M, et al. (2019) Exploiting the allelopathic potential of aqueous leaf extracts of Artemisia absinthium and *Psidium guajava* against Parthenium hysterophorus, a widespread weed in India. Plants 8(12): 552.

31. Barkatullah SN, Khushnood Ur Rehman, Butt ZA, Yaseen T, Akbar K, et al. (2021) Allelopathic potential of Indian plum (*Ziziphus mauritiana L.*) Contrary to wheat (*Triticum aestivum L.*), maize (*Zea mays l.*) And chickpea (*Cicer arietinum L.*,). Pak J Weed Sci Res 27(1): 23-34.

32. Elaloui M, Ghazghazi H, Ennajah A, Ben Youssef I, Ben Othman N, Laamouri A (2017) Allelopathic effects of *Ziziphus jujuba* and *Z. lotus* leaf extracts on Triticum durum and Lens culinaris.

33. Hossain MM, Miah G, Ahamed T, Sarmin NS (2012) Study on allelopathic effect of Moringa oleifera on the growth and productivity of mungbean. International Journal of Agriculture and Crop Sciences 4(15): 1122-1128.

34. Poonpaiboonpipat T, Krumsri R, Kato-Noguchi H (2021) Allelopathic and Herbicidal Effects of Crude Extract from *Chromolaena odorata* (L.) RM King and H. Rob. on Echinochloa crus-galli and *Amaranthus viridis.* Plants 10(8): 1609.