Allelopathic Effects of Sweet Basil (*Ocimum basilicum* L.) on Seed Germination and Seedling Growth of Pearl Millet (*Pennisetum glaucum* L.)

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Abstract

Ocimum basilicum (O. basilicum) L. *(sweet basil) is a traditional medicinal plant, well known for its variety of therapeutic properties and its abundant bioactive secondary metabolites, while* Pennisetum glaucum (P. glaucum) *(pearl millet) is a drought-tolerant cereal of global economic importance. The present study investigated the allelopathic potential of* O. basilicum *leaves and seeds on the seed germination and early seedling growth of* P. glaucum *under laboratory and greenhouse conditions. The experiments were both completely randomized with 3 replications. The aqueous extracts were obtained and tested on the seeds of* P. glaucum *and morphological parameters of germination and growth of the germinated seedlings were determined after 3 days. It significantly ($P < 0.05$ to $P < 0.001$) inhibited germination and growth in a concentration-dependent manner. In the laboratory bioassay, dry-seed extract at 30 g reduced germination to 56 % (versus 100 % in the control), radicle length to 1.15 cm (versus 2.67 cm) and plumule length to 1.25 cm (versus 3.05 cm). Fresh-leaf extract produced milder but still significant reductions. In the greenhouse experiment, the powdered leaves and seeds were added to the soil at rates of 10, 20 and 30 g pot-1 and the growth responses of the plants were measured 30 days after sowing on the basis of plant height, root length, number of leaves and fresh and dry biomass. In the greenhouse, 30 g seed powder lowered germination to 74 %, plant height to 6.30 cm (versus 12.33 cm), leaf length to 2.87 cm and root length to 2.90 cm, with corresponding declines in fresh and dry biomass. The highest concentration (30 g) recorded the highest germination percentage reduction, plant height, root length and biomass reduction compared with the untreated control.* O. basilicum *has a high allelopathic potency to inhibit the germination and early growth of* P. glaucum*. The results suggest that* O. basilicum *possesses promising allelopathic properties.*

Keywords: Allelopathic Effects, *O. Basilicum*, *P. glaucum*, Dry Seeds and Fresh Leaves Extracts, Plumule and Radicle Length and Biomas

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Introduction

Plants in the *Ocimum* genus has been identified as potential heterotrophic species (Balicevic et al., 2015) One plant might have a direct or indirect negative effect on other plants by releasing chemical components into the surrounding (Delabays et al., 2004). Allelochemicals are chemical compounds produced by plants that can have varying effects on the growth of other plants. These chemicals can promote growth at low concentrations but inhibit growth at higher concentrations (Gulzar et al., 2016; Ishak and Sahid, 2014; Mushtaq, 2018 Mushtaq et al., 2019; Gholami et al., 2011). Allelochemical can interfere plant growth and absorb nutrients. The degree of inhibition increases as the concentration of allelochemicals increases. Additionally, auxin promotes shoot and root growth, but allelochemical can prevent cell division. It is important to note that the growth inhibition may be caused by the potential of this allelochemical to interfere with plant growth systems (Gholami et al., 2011). Millet *Pennisetum glaucum* (*P. glaucum*) is a cereal crop that is increasingly being used as an alternative to straw cultivation for ground cover in no-till farming. It is known for its drought tolerance, wide adaptability, and good agronomic properties, which make it suitable for mass production. It has the advantages of rapid growth, low cost of implementation, and good quality food. It is widely cultivated in Brazil, particularly in the Cerrado region, and it is a good option for system integration. It is often grown alongside legumes, which are also important crops in Brazil. Millet is known for its ability to grow well in dry conditions and is often used as a staple food in many parts of the world (Priesnitz et al., 2011).

The commercial cultivation of *Ocimum basilicum* (*O. basilicum*). for the extraction of essential oils typically takes place during the kharif season, which is the period from June to September when weed activity is low. We study to investigate the allelopathic properties of *O. basilicum* by using different plant parts, such as leaves and seeds, and by varying the concentration of phytotoxic materials. The aim of this study was to comprehensively investigate the allelopathic properties of *O. basilicum*, focusing on its ability to inhibit the growth of *P. glaucum* through various stages including seedling growth, germination, and survival. Recent research has examined the herbicidal potential of Ocimum-derived materials and the phytotoxic activity of linalool and eugenol from Ocimum species (Anupama et al., 2023; Verma et al., 2026).

Although the allelopathic activity of *O. basilicum* has been reported against several weed and crop species, previous studies have mainly focused on single plant parts, limited bioassays, or only laboratory evaluations. A comprehensive comparison of the allelopathic effects of

both fresh leaf and dry seed extracts of *O. basilicum* on *P. glaucum* under both laboratory and greenhouse conditions remains lacking. Moreover, limited information is available on linking the observed phytotoxic effects with the identification of bioactive allelochemicals through GC–MS analysis. Therefore, the present study addresses these gaps by comparing the allelopathic potential of fresh leaves and dry seeds of *O. basilicum* across different concentrations, evaluating their effects on seed germination, seedling growth, biomass production, and vigor under controlled laboratory and greenhouse conditions, and identifying the major allelochemicals responsible for the observed phytotoxicity using GC–MS analysis. Recent syntheses have emphasized distinguishing controlled phytotoxicity from allelopathy established in natural settings (Shadab et al., 2024; Talarico et al., 2026).

The specific objectives of this allelopathic study are as follows: Evaluation of the allelopathic effects of fresh leaf and dry seed extracts of *O. basilicum* on the seed germination and early seedling growth of *P. glaucum* under laboratory conditions; Assessment of the effects of incorporating *O. basilicum* leaf and seed powders into the soil on the growth and biomass of *P. glaucum* under greenhouse conditions; Comparison of the phytotoxic potential of different concentrations of leaf and seed treatments; Identification of the major allelochemicals present in *O. basilicum* leaves and seeds using GC–MS analysis and relate them to the observed allelopathic activity.

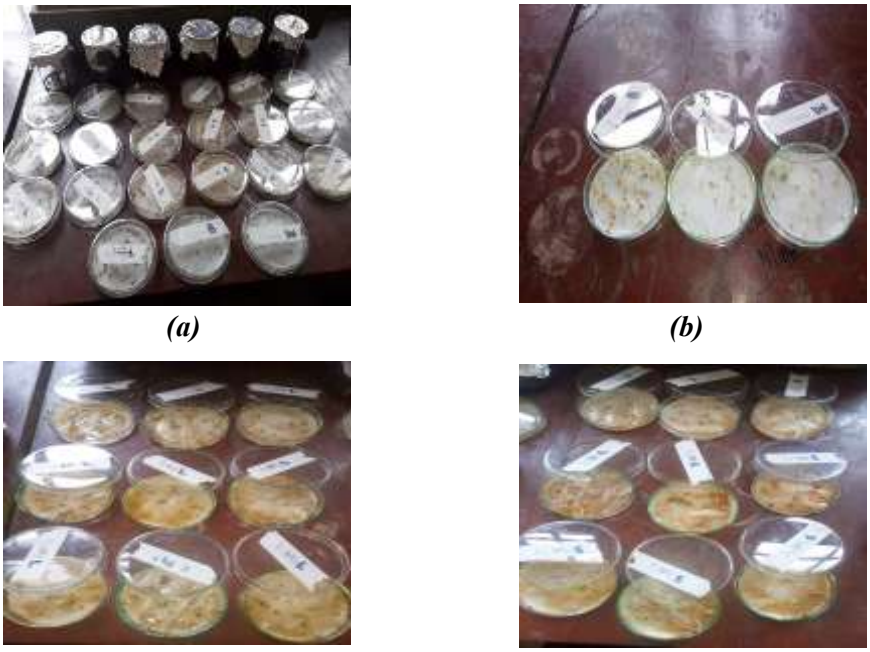
Material and Methods

This study aimed to investigate the allelopathic effects of aqueous fresh leaf and dry seed extracts of *O. basilicum* on the germination, radicle length band plumule length of *P. glaucum*. The method used in this experiment was based on the method described by Amoo et al., 2008.

Collection

The mature leaves and seeds of *O. basilicum* were harvested from the University of Peshawar, Department of Botany, Pakistan. The leaves were washed and homogenized in a sterile mortar and pestle to get a uniform plant material. To remove surface impurities, the seeds were washed first with tap water and then with distilled water and were air dried at room temperature (14-20 °C). Each 100 mL of distilled water was used to extract the 10, 20 and 30 g of the fresh leaves or seeds respectively. The mixtures were left to extract at ambient conditions and the extracts were filtered to remove plant debris. For 72 h, the filtrates were kept at 5–10 °C without damaging the biological activity. Concentration of 10, 20 and 30 g were selected to represent low, medium, and high allelopathic exposure

levels and were based on concentration ranges commonly employed in previous allelopathy studies evaluating dose-dependent phytotoxic effects of aqueous plant extracts on seed germination and seedling growth (Figure 1) (Muhammad et al., 2019; Ajribzadeh et al., 2017).



(a) ***(b)***

(c) ***(d)***

Figure 1: (a) Three Raplicates of fresh leaves and dry seed extracts having concentration (i-e 10 g, 20 g and 30g) with three Raplicates of control; (b) Three Raplicates of control tratment after 72 hours; (c) Germination in three replicates of differen t concentration (i-e; 10 g, 20 g, and 30g) of* O. basilicum *Leaves extracts after 72 hours; (d) Germination in three replicates of different concentration (i-e; 10 g, 20 g, and 30g) of* O. basilicum *after 72 hours

Standard Filter Paper Bioassay

After sterilizing the petri dishes for this experiment, Whatman #1 filter paper and cotton were applied as seedbeds. The cotton was first spread out in Petri dishes and then a double-layer of Whatman No. 1 filter paper was placed on top. The required plant extracts (such as leaves and seeds) were applied to the seedlings on this filter paper for the test, and distilled water for the control. Excess extract or distilled water was severely prohibited to decrease the effects of water-logging on germination and seedling growth. Healthy and uniform seeds were always selected for each experiment. The seeds were kept equidistantly within the

Petri dishes. To maintain humidity and moisture in the Petri dishes, they were constantly covered with larger Petri dishes. Petri dishes and their contents were incubated at 26°C. The germination percentage, shoot and root length of individual seedlings were measured with the help of ruler after 72 hours.

Extract Bioassay

Mature and fresh leaves and seeds in dry form of *O. basilicum* plant were collected and brought to laboratory, Department of Botany, Peshawar University, where the fresh leaves were crushed by using Mortar and pestle while dry seeds were powdered using the electric grinder. Powdered seeds and fresh leaves of *O. basilicum* were taken three times and soaked individually in 100 ml of water for 24 and 48 hours at 26°C before filter to obtain the extracts of both plant parts. By using the "standard filter paper bioassay," these extracts were then tested on *P. glaucum* as the test species (Barkatullah et al., 2010; Hussain et al., 2010). Distilled water or extracts were used for control. Ten seeds per replicate were used for the test and control treatments. The incubator was set at 26°C for Petri dishes. After 72 hours, fresh and dry seedling weights and germination were reported.

Greenhouse Experiment

The current experiments were performed in the greenhouse of the University of Peshawar's botanical garden in Pakistan to investigate the allelopathic impacts of *O. basilicum* leaves and seeds on the growth of *P. glaucum* plants. The leaves and seeds powder of sweet basil was mixed in per kg soil at concentrations of 10 g, 20 g, and 30 g on a w/w in pots. Each treatment involved three pots with a diameter of 20 cm, and a control group was also established with three pots. *P. glaucum* 5 seeds were buried in every pot. Treatments were organized in a completely randomized design with three replicates for each crop. After the seeds had germinated, thinning was performed to maintain five plants per pot. The pots were watered regularly. The experiments were concluded 30 days after sowing, and various measurements were taken, including the height of the plants (cm), the eaves and root length (cm), and the fresh and dry weights of the seedlings in grams (Sharma et al., 2016).

Standard Germination

The percentage of the test species' germination was reported by using the following formula (Scott et al., 1984):

$$\text{Germination } \% = \frac{\text{Germinated seed}}{\text{Total no.of seed}} \text{ x } 100$$

Plumule and Radicle Length in cm

The study involved measuring the plumule and radicle lengths of 30 seedlings per treatment. These seedlings were selected randomly from three replication groups. A measuring scale was used to take the measurements of the plumule and radicle length.

Statistical Analysis

The results of each experiment is the mean of three replicates are performed in a completely randomized block design. At alpha = 0.05, all data were statistically determined to be significantly different from the control using one-way ANOVA. The average and standard deviation of three replicates is used to express all the data.

Inhibition Percentages

Different inhibition percentages such as, Germination inhibition %, inhibition % of shoot and root length, inhibition % of seedling growth were calculated by the following formulas (Belz et al., 2022; Eisler, 2000).

Germination Inhibition Percentage **(Belz et al., 2022)**

$$\text{Germination inhibition \%} = \frac{\text{Germination \% of the treatment} - \text{germination \% of the control}}{\text{Germination \% of the control}} 100$$

Inhibition % of Root Length (Eisler, 2000)

$$\text{Inhibition \% of root length} = \frac{\text{Root length of the treatment} - \text{root length of the control}}{\text{Root length of the control}} 100$$

Inhibition % of Shoot and Seedling Length (Macías et al., 1998; Uremis et al., 2009)

$$\text{Inhibition \% of shoot length} = \frac{\text{Shoot length of the treatment} - \text{shoot length of the control}}{\text{Shoot length of the control}} 100$$

$$\text{Inhibition \% of seeedling length} = \frac{\text{Seedling length of the treatment} - \text{sheedling length of the control}}{\text{Shoot length of the control}} 100$$

Vigor Indices

Vigor indices of root, shoot and seedling were estimated as per formulas ((Alsaadawi et al., 2019; Lemerle et al., 1996).

Shoot Vigor Index **(Alsaadawi et al., 2019)**

Shoot vigor index = Germination % × shoot length

Root Vigor Index (Lemerle et al., 1996)

Root vigor index = Germination % × root length

Seedling Vigor Index (Lemerle et al., 1996)

Seedling vigor index = Germination % × seedling length

GCMS

Samples were prepared by extracting substances from basil leaves using a solution of methanol in a ratio of 1:10, 1-part leaves to 10 parts methanol. The extracted solution obtained from the leaves was then analyzed using a combined Gas Chromatography-Mass Spectrometry (GCMS) system at the CRL (Chemical Research Laboratory) at the University of Peshawar. The GCMS method allowed to match mass spectra and relative retention times to qualitatively assign names to the compounds under study (RTs). Utilized search databased of mass spectroscopy, namely NIST 1998 and GCMS Library Shimadzu 1996. This comprehensive approach enabled us to found the presence of active constituents in each sample. This process aimed to identify specific chemicals known as allelochemicals present in the basil leaves and seeds.

Results

Laboratory Experiment

Percent Germination (%): The aqueous extract of dry seeds and fresh leaves of *O. basilicum* reduced the germination % of *P. glaucum*. After 72 hours' incubation period the highest inhibitory effect was found as (56%) of dry seeds extract at 30 g treatment compared with the control was recorded (100%), while the lowest inhibitory impact (70%) was seen at a concentration of 10 g, respectively. Furthermore, the results showed that the minimum percent germination for fresh leaves extract was (75%) at 30 g, while the maximum was observed (90%) at 10 g of aqueous leaf extract in comparison with control. In comparison to fresh leaves extract and control, the results also demonstrated that aqueous extract of dry seeds had a greater inhibitory effect on germination percentage (Table 1).

***Table 1: Allelopathic effect of fresh leaves and dry seeds extract of* O. basilicum *on percent germination of* P. glaucum**

Treatments	Control	10 g	20 g	30 g
Effect of Fresh Leaves Extract	100±0.00	90±1.15	86±1.22	75±1.16
Effect of Dry Seeds Extract	100±0.00	70±1.19	65±1.21	56±1.13

After statistical analysis, the results evidently indicated that all applications of extracts from fresh leaves and dry seeds of *O. basilicum*

significantly reduced the root length of *P. glaucum*. Extract obtained from 30 g of dry seeds was more inhibitory for radicle length, reducing radicle length to (1.15±0.08 cm), followed by 20 g dry seeds extract to (1.22±0.06 cm) as compared to control (2.67±0.22 cm). The results also revealed that 10 g fresh leaves extract was less inhibitory for the under mentioned parameter that reduced the radicle length to (2.29±0.25 cm), followed by 20 g concentration which was, (2.07±0.26 cm) as compared to control (Table 2; Figure 2).

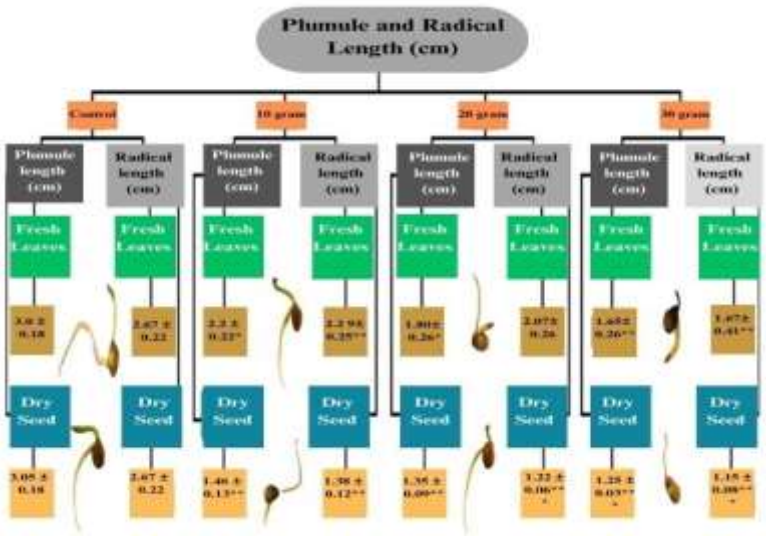


***Figure 1: Effect of different concentration of fresh leaves and dry seeds extracts of* O. basilicum *on Plumule and Radicle Length of* P. glaucum**

***Table 2: Allelopathic effect of fresh leaves and dry seeds extract of* O. basilicum *on Radicle Length of* P. glaucum**

Treatments	Control	10 g	20 g	30 g
Effect of Fresh Leaves Extract	2.67±0.22	2.29±0.25*	2.07±0.26*	1.67±0.41**
Effect of Dry Seeds Extract	2.67±0.22	1.38±0.12**	1.22±0.06***	1.15±0.08***

*The results are categorized by the level of significance, where * P < 0.05; ** P < 0.01; *** P < 0.001

Plumule Length (cm): According to statistical analysis, all applications of fresh *O. basilicum* leaves and dry seeds extract significantly reduced the plumule length of *P. glaucum* seedlings. The data revealed that 30 g dry seeds treatment has more significantly suppressed the plumule length (1.25±0. 03 cm) of *P. glaucum* followed by 20 g seeds extracts (1.35±0.09 cm) as compared to control. The result also showed that 10 g and 20 g fresh leaves extract was less inhibitory for the under mentioned parameter that reduced the plumule length to, 5 g (2.23±00.22 cm) and 10 g to (1.80±0.26 cm), in comparison with control (3.05±0.18

cm). The result showed that all the treatments among dry seeds extract were significantly inhibit ed the plumule length of *P. glaucum* as compared to fresh leaves extract and control (Table 3).

***Table 3: Allelopathic effect of different concentration of fresh leaves and dry seeds extracts of* O. basilicum *on Plumule Length of* P. glaucum**

Treatment	Control	10 g	20 g	30 g
Effect of Fresh Leaves Extract	3.05±0.18	2.23±0.22*	1.80±0.26*	1.65±0.26**
Effect of Dry Seeds Extract	3.05±0.18	1.46±0.13***	1.35±0.09***	1.25±0.03***

(* P < 0.05; ** P < 0.01; *** P < 0.001)

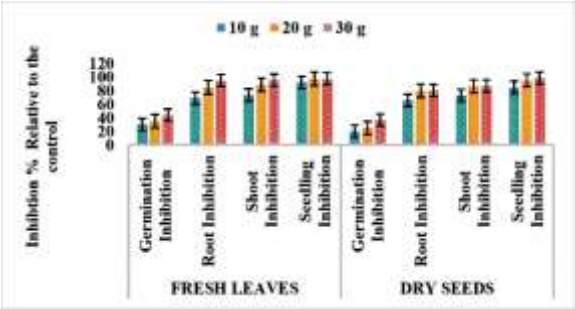


Figure 2: Inhibition Concentration (%) of* O. basilicum *Leaves and seed Aqueous Extract on P. glaucum

Biomass (gm): The current result of above table finds out that both fresh leaves and dry seeds aqueous extract of *O. basilicum* significantly reduce the biomass of the *P. glaucum*. The maximum standards of fresh weight were 1.2 g and 1 g for the aqueous extracts of seeds and leaves with respect to the control, while the respective values of dry weight were 0.43 g and 0.38 g. The maximum fresh weight value of 1.27 g was found in 10 g of fresh leaf extract, while the maximum reduction of 0.83 mg was found in 30 g of dry seed extract. Similarly, the maximum value of dry weight for the aqueous extract of leaves, 0.53 g, was found at a concentration of 10 g, while the minimum values of dry weight for the aqueous extracts of leaves and seeds were 0.35 g and 0.28 g, respectively, at a concentration of 30 g. These results showed that with increasing concentration, the dry and fresh of *P. glaucum* decreased significantly ($p \leq 0.05$).

The study in Figure 4 evaluated the impact of aqueous extracts of seeds and leaves of *O. basilicum* on the growth of the *P. glaucum*. by quantifying the growth inhibition relative to a control group. The results showed that the highest extract concentration had the most significant negative effect on growth of seeds and the four parameters of germination percentage, root and shoot length, and seedling growth inhibition showed consistent responses. as well as the vigor indices, which were also calculated by multiplying the germination percentage with seedling

growth traits (root and shoot and seedling lengths), and found that these indices showed a significant decline in shoot, root, and seedling growth when seedlings were subjected to irrigation with high extract concentrations (10 g, 20 g, and 30 g) (Figure 4). However, the control treatment and the lowest extract concentration (10 g) did not show any significant differences in these indices.

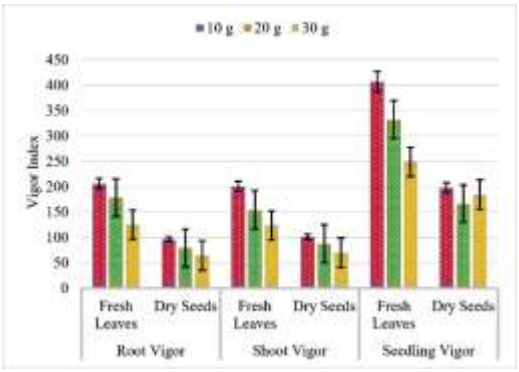


Figure 3: Vigor indices such as root, shoot, and seedling growth of* P. glaucum *subjected to the aqueous leaves and seed extract of* O. basilicum *at different concentration levels

Effects on Biomass (Fresh and Dry Weight)

The highest dry and fresh weight of *P. glaucum* were recorded in the control treatments after 30 days of planting. When we incorporated 30g or more of powdered of leaves and seeds of sweet basil into the soil, it significantly reduced the fresh and dry weight of *P. glaucum.* compared to the control treatments. Furthermore, the reduction in dry and fresh weight increased as the concentrations of powdered leaves and seeds in the soil increased. The incorporation of powdered sweet basil leaves and seeds into the soil inhibited dry weight at 1.7 g, while fresh weight showed less inhibition at 3.8 g. Both fresh and dry weights were significantly affected by the application of both plant part extracts, with fresh weight being less affected compared to dry weight (Table 4).

***Table 4: Allelopathic effect of dry seeds and fresh leaves extract of* O. basilicum *on fresh and dry weight of* P. glaucum**

Treatments	Fresh weight		Dry weight	
	Effect of Fresh Leaves Extract	Effect of Dry Seeds Extract	Effect of Fresh Leaves Extract	Effect of Dry Seeds Extract
Control	1.42	1.42	0.73	0.73
10 g	1.27*	1.24**	0.53***	0.49***
20 g	1.22*	0.93**	0.41***	0.38***
30 g	1.11*	0.83**	0.35***	0.28***
Mean	1.2	1	0.43	0.38

(* P < 0.05; ** P < 0.01; *** P < 0.001)

Greenhouse Experiment

Pots experiment was performed in greenhouse, Department of Botany, University of Peshawar, which indicated that the inclusion of sweet basil leaves and seed powder in the soil significantly ($P \leq 0.05$) reduced the growth of *P. glaucum* seedlings compared to the control group (Tables 5).

Table 5: Allelopathic effect of leaves and seeds powder of* O. basilicum *into the soil on Germination % and plant height of* P. glaucum *in pot experiment

Treatment	Germ. (%) Leaves	Germ. (%) Seeds	Height (cm) Leaves	Height (cm) Seeds
Control	100±0.0	100±0.0	12.33±2.61	12.33±2.61
10 g	93±0.82	90±0.82	9.77±0.69*	8.60±0.73*
20 g	87±1.25	84±0.94	7.33±2.11**	7.47±1.85**
30 g	76±2.87	74±1.41	6.10±1.30***	6.30±2.30***

(* P < 0.05; ** P < 0.01; *** P < 0.001)

Effects on Germination Percentage and Plant Height

After 30 days of planting, the control group showed the highest germination percentage and plant height in *P. glaucum* seedlings. However, increasing the concentration of powdered sweet basil leaves and seeds in the soil resulted in the lowest plant height for *P. glaucum* seedlings. The powder of leaves and seeds when incorporated into the soil at concentrations of 10 g, 20 g to 30 g decreased the germination percentage of *P. glaucum*. The highest inhibition, at 74±1.41%, occurred with a 30g concentration when we applied dry seed powder, while the lowest inhibition, at 90±0.82, was observed with a 10g concentration compared to the control treatments at 100±0.0% (Table 7). Regarding plant height, the most significant reduction occurred when we incorporated the powder at a concentration of 30g. At a high concentration of powdered leaves and seeds, the plant heights were significantly ($P \leq 0.05$) decreased to 6.10±1.30 cm by leave powder, followed by 30g seed powder (6.30±2.30cm) in *P. glaucum* seedlings while, minimum plant height was observed in 10 g leaves powder in to the soil (9.77±0.69 cm) as compared to control (12.33±2.61 cm). Furthermore, the reduction in germination percentage and plant height increased as the rate of powdered leaves and seeds in the soil increased.

Table 7: Allelopathic effect of leaves and seeds powder of* O. basilicum *into the soil on Fresh and Dry Weight of* P. glaucum *in pot experiment

Treatment	Fresh weight		Dry weight	
	Effect of leaves	Effect of seeds	Effect of leaves	Effect of seeds
Control	4.5	4.5	3.7	3.7
10 g	3.8*	3.5*	3.1*	2.9**
20 g	3.3*	2.8**	2.7**	2.5**
30 g	2.9**	2.3***	1.9***	1.7***

(* P < 0.05; ** P < 0.01; *** P < 0.001)

Effects on Leaves and Root Length

Incorporating the powder of leaves and the seed of sweet basil into the soil significantly ($P \leq 0.05$) reduced the length of root and leaves of *P. glaucum* seedlings. The reduction in number of root and leaves length s increased with the concentration of the powdered leaves and seeds in the soil. At 30 days after planting, the *P. glaucum* number of leaves and root showed the highest length in the control treatment, measuring 7.47±1.03 and 6.27±0.55 cm, respectively. Maximum inhibition in the number of leaves was observed in 30 g at 2.87±0.54 cm, while, minimum reduction was found in 10 g at 5.93±1.19 as compared to control group. Furthermore, the highest inhibition was observed in root length at 2.9±0.70 cm, while the lowest inhibition was found in 10 g of leaves powder at 4.27±0.35cm compared to the control. The most significant reduction occurred when we incorporated the powder at a concentration of 30g, while the least reduction was found at 10g and 20g concentrations, respectively. However, the overall results showed that the inhibition of number of leaves and root lengths depended on the concentration (Table 6, Figure 5).

Table 6: Allelopathic effect of seeds and leaves powder of* O. basilicum *into the soil on number of leaves and root length P. glaucum in pot experiment.

Treatment	Leaves Length (cm)		Roots Length (cm)	
	Leaves	Seeds	Leaves	Seeds
Control	7.47±1.03		6.27±0.55	
10 g	5.93±1.19*	4.93±0.95*	4.27±0.35*	4.03±0.51*
20 g	4.17±0.63**	3.67±0.66**	3.83±0.50**	3.23±0.38**
30 g	3.1± 0.59***	2.87±0.54***	3.03±0.29***	2.9±0.70***

(* P < 0.05; ** P < 0.01; *** P < 0.001)

GCMS and Reported Allelochemical

Capillary GCMS is used to separate, recognize and measure allelochemicals (An et al., 1996). The GCMS data indicated the prevalence and potential significance of these compounds as allelochemicals, suggesting their role in plant interactions within their environment. The variation in abundance percentages might imply different levels of impact or effectiveness in influencing neighboring organisms or ecological processes (Table 8, 9).

In current study GCMS analysis of *O. basilicum* leaves revealed the presence of several and different constituents. Among these compounds, the predominant ones include Estragole (41.59% abundance), It's an aromatic compound often found in essential oils, contributing to the characteristic aroma of basil. However, it's worth noting that estragole has been a subject of regulatory concern due to potential of allelopathy at higher concentrations. β-Terpinene (10.99% abundance), this compound adds to the fragrance of basil and is commonly found in various plant

essential oils. α-Pinene (6.11% abundance) is aromatic compound, α-pinene, contributes to the distinct aroma associated with basil leaves. α-Caryophyllene (7.33% abundance) is known for its spicy and peppery aroma, α-caryophyllene is a common terpene.

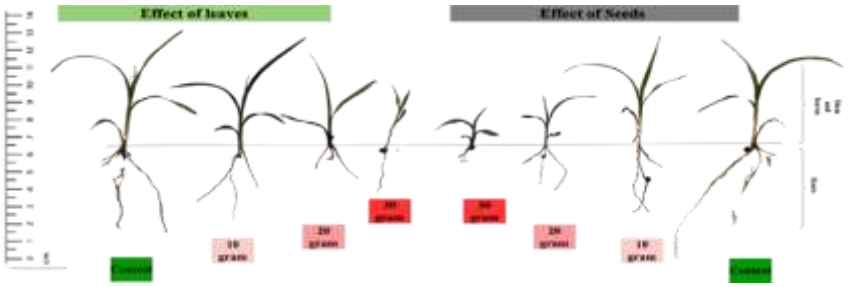


Figure 4: Randomly selected plants of pots experiment for examined length of its shots and roots

***Table 8: GCMS and allelochemical of Leaf extract of* O. basilicum**

RT	Area (%)	Compound	IUPAC name	MF	MW	Reference
7.02	7.13	Eucalyptol	(1s,4s)-1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane	C10H18O	154.14	Puig et al. (2018)
8.99	10.99	β -terpinene	1-isopropyl-4-methylenecyclohex-1-ene	C10H16	136.13	Puig et al. (2018), Requesón et al. (2022)
10.98	6.11	α-Pinene	2,6,6-trimethylbicyclo [3.1.1] hept-2-ene	C10H16	136.13	Puig et al. (2018)
11.69	41.59	Estragole	1-allyl-4-methoxybenzene	C10H12O	148.09	Requesón et al. (2022)
13.01	7.99	p-mentha-1(7), 8-diene	1-methylene-4-(prop-1-en-2-yl) cyclohexane	C10H16	136.13	Gowda (2023)
13.97	3.67	Methyl-2-phenyl-prop-2-enoate	methyl 2-phenylacrylate	C10H10O2	162.07	-
15.07	3.59	Eugenol	4-allyl-2-methoxyphenol	C10H10O2	164.08	Govêa et al. (2020), Choopayak et al. (2022),
16.51	7.03	(E)-methyl cinnamate	methyl cinnamate	C10H10O2	162.07	Lorenzo et al. (2019)
18.12	4.56	Cubebene	(3aS,3bR,4S,7R,7aR)-4-isopropyl-7-methyl-3-methyleneoctahydro-1H-cyclopenta [1,3] cyclopropane [1,2] benzene	C15H24	204.19	Kato-Noguchi & Kato, 2022
20.13	7.33	α-Caryophyllene	(1R,9S, Z)-4,11,11-trimethyl-8-methylenebicyclo [7.2.0] undec-4-ene	C15H24	204.19	Wang et al., 2014

*RT: Retention Time; % A: Area; MF: Molecular Formula; MW: Molecular Weight

These compounds are characteristic constituents of basil leaves and contribute not only to their aroma but also potentially to their therapeutic properties. However, the prominence of estragole raises concerns due to its potential health risks at higher concentrations.

The diverse range of compounds found in basil seed extract suggests its potential allelopathic effects. The presence of compounds like

eucalyptol, eugenol, germacrene, linalool format and menthol, known for their biological activities, might imply the role of basil in influencing neighboring organisms or ecological interactions.

***Table 9: GCMS and allelochemical of Leaf extract of* O. basilicum**

RT	Area (%)	Compound	IUPAC name	MF	MW	Reference
7.22	4.67	Eucalyptol	(1s,4s)-1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane	C10H10O	154.14	Puig et al. (2018)
7.39	5.11	1,3,6-octatriene, 3,7-dimethyl-, (e)-	(E)-3,7-dimethylocta-1,3,6-triene	C10H16	136.13	El-Kareim et al. (2021)
8.19	41.1	Eugenol	4-allyl-2-methoxyphenol	C10H10O2	164.08	Govêa et al. (2020), Choopayak et al. (2022)
12.79	12.07	Germacrene	(3R,6S,9R, Z)-6-isopropyl-3,9-dimethylcyclodec-1-ene	C15H28	208.22	Miranda et al., 2014
12.89	6.1	Cyclohexanethanol,4-ethenyl-	2-(4-vinylcyclohexyl) ethan-1-ol	C10H180	154.14	-
13.78	2.55	1-propene, 3-bromo-	3-bromoprop-1-ene	C3H5Br	119.96	-
16.19	3.15	Beta elemene	(1S,2R,4S)-1-methyl-2,4-di(prop-1-en-2-yl)-1-vinylcyclohexane	C15H24	204.19	-
18.18	2.24	9-dodecen-1-ol, (z)-	(Z)-dodec-9-en-1-ol	C12H24O	184.18	-
20.75	4.26	Bis-(3,5,5-trimethylhexyl) ether	3,5,5-trimethyl-1-((3,5,5-trimethylhexyl) oxy) hexane	C18H38O	270.29	-
21.73	6.37	Linalool formate	3,7-dimethylocta-1,6-dien-3-yl formate	C11H18O2	182.13	Nebapure & Srivastava (2019)
24.17	5.92	Menthol	2-isopropyl-5-methylcyclohexan-1-ol	C10H20O	156.15	Xiao et al. (2017), Ouyang et al. (2020)
24.44	6.78	Methyl(z) cinnamate	methyl (Z)-3-phenylacrylate	C10H10O2	162.07	Lorenzo et al. (2019)

*RT: Retention Time; %A: Area; MF: Molecular Formula; MW: Molecular Weight)

The findings from the GCMS analysis (Figures 6 & 7) reveal that extracts derived from basil seeds and leaves contain a significant amount of allelochemicals. Hence these finding supports the idea that the allelopathic properties of plants primarily stem from these specific compounds.

The current analysis provides valuable insights into the chemical composition of basil seed extract and its potential implications in allelopathy, offering a basis for further studies on the role of basil in its ecosystem and its potential applications in agriculture or natural resource management.

Effect of Allelochemical

Allelochemicals are naturally occurring secondary metabolites of plants that affect the germination, growth and development of other plants in the area through an allelopathic effect. Of these compounds, phenolics, terpenoids, flavonoids and other bioactive metabolites have been commonly reported as phytotoxic compounds that inhibit root and shoot growth by interfering with the essential physiological processes (Hussain and Reigosa, 2011).

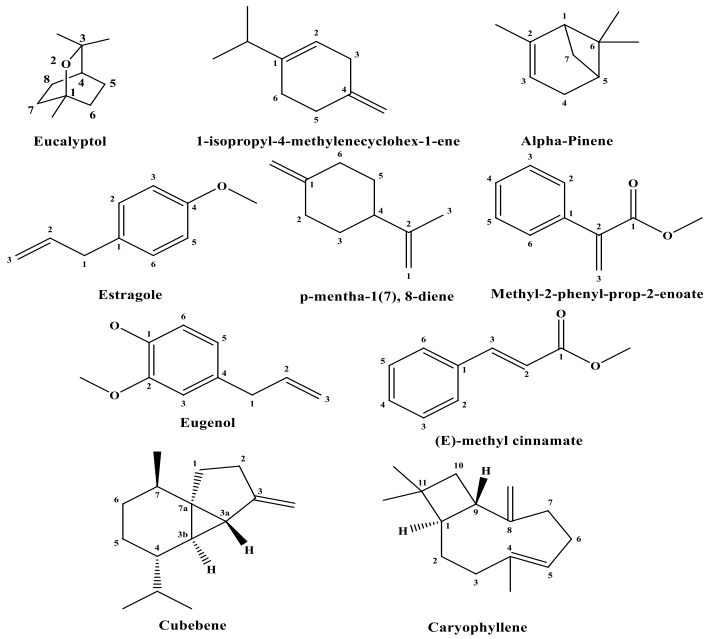


Figure 5: Structure formula of compound of* O. basilicum *leaves and seeds extract

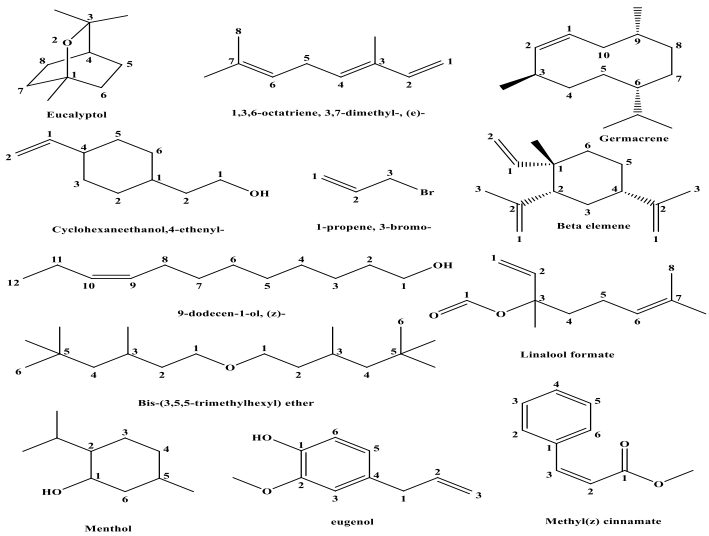


Figure 6: Structure formula of compound of* O. basilicum *leaves and seeds extract

Mitochondrial dysfunction is one of the key mechanisms for allelopathic inhibition. Weir et al. (2004) have reported that certain

monoterpenes such as camphor, α-pinene and limonene are reported to interact with mitochondrial membranes to inhibit the respiration of soybean radicle and hypocotyl tissues, but each compound seems to do so through different biochemical pathways. A next step in allelochemical disruption is the reduction of plant growth and vigor that occurs through the disruption of a variety of metabolic processes, such as photosynthesis, nutrient acquisition, enzyme activity, membrane integrity and hormonal imbalance, as a result of those allelochemicals' impact on cellular respiration (Macías et al., 2007).

Generally, the biological effect of allelochemicals is concentration-dependent and is species-specific, depending on the sensitivity of the recipient species. Some allelochemicals can promote plant growth at low concentrations, while higher concentrations can have phytotoxic effects, which can reduce germination and establishment of seedlings. In addition, the allelochemicals are known to induce changes in plant defensive metabolism when neighboring plants are present, which can also alter the synthesis and release of allelochemicals. Such dynamic chemical interactions are crucial in controlling plant competition, plant community structure and ecosystem function. Therefore, knowledge of allelopathy mechanisms offers good insights into the development of environmentally-friendly strategies for weed control and crop cultivation (Broz et al., 2010) (Figure 8).

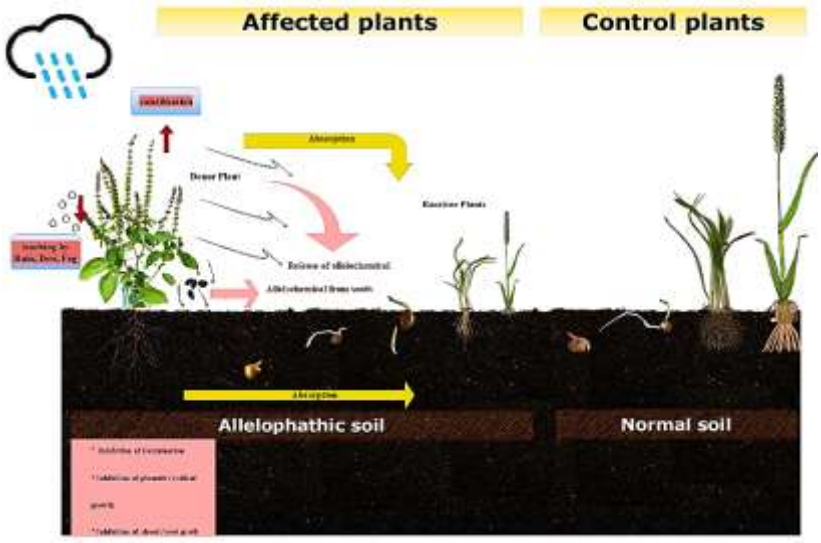


***Figure 7: Over all mechanism of allelopathy of* O. basilicum *on* P. glaucum**

Discussion

In the present study, the allelopathic effects of aqueous leaf and seed extract of *O. basilicum* on the germination and early seedling growth of *P. glaucum* were studied in laboratory and greenhouse conditions. The results were quite clear that both extracts significantly reduced the seed germination and fresh and dry biomass accumulation; plumule and radicle elongation was also significantly reduced. The inhibitory response was also dose dependent, with increasing concentrations of the extracts resulting in increasing drop in all the growth parameters. *P. glaucum* showed significant reduction in germination percentage, radicle length, plumule length and biomass after 48 hr exposure to leaf and seed extracts of *O. basilicum* under laboratory conditions. The results are in line with those of Sharma and Singh (2004) who reported that basil has significant allelopathic potential to affect the germination and establishment of seeds of various weed species. The present findings are consistent with those reported by Asad et al. (2026), who demonstrated that extracts of Albizia lebbeck significantly inhibited seed germination and seedling growth of Hordeum vulgare, Zea mays and Phaseolus vulgaris. Their study attributed the phytotoxic effects to bioactive allelochemicals present in the plant extracts, supporting the concept that plant-derived secondary metabolites can suppress the growth and development of neighboring species. Similarly, the concentration-dependent inhibition observed in the present study suggests that allelochemicals present in *O. basilicum* contribute to its phytotoxic activity. The study showed that addition of basil leaf powder to the soil affected the emergence of species like red foxtail, beggar's scab, guinea grass, and radish, but not the water-treated control; aqueous basil leaf extract resulted in a significant reduction in seed germination compared with the water-treated control. Both extracts (leaf and seed) had inhibitory activity but there was a difference in phytotoxicity level between the extracts, the leaf extracts being more phytotoxic than the seed extracts. The allelopathic effect of the leaves, roots and seeds of *O. basilicum* on economically important crops such as mustard, wheat, barley, okra, lentil and pea were also studied by Đikić (2005), Dhima et al. (2009) and Verma et al. (2012). The results were always that all plant extracts inhibited germination and seedling growth and that the leaf extracts had the most inhibitory effect on germination followed by the root extracts and the seed extracts. Comparable concentration-dependent inhibitory responses have been reported in controlled seed-germination and seedling-growth assays involving other plant aqueous extracts (Zhao et al., 2025; Ahmadnia et al., 2026).

The higher phytotoxicity of leaf extracts in the current study corroborates the previous research with other allelopathic plants species.

The leaf extracts of P. hysterophorus have shown higher inhibition of seed germination than the other parts of the plant according to Maharjan et al. (1970). Similar conclusion was drawn by Kanchan and Jayachandra (1980) and Tefera (2002) who showed that the leaves of P. hysterophorus contain more allelochemicals which inhibit germination and seedling growth. Similarly, Srivastava et al. (1985) found extracts of leaf and flower material to be effective in inhibiting germination and seedling growth of pea, barley and wheat. The phytotoxic effects observed in the present study may also be associated with physiological disturbances induced by plant-derived compounds. Shahid et al. (2023) reported that exogenous serine significantly influenced plant growth and biochemical constituents under arsenic stress, highlighting the importance of bioactive compounds in regulating plant physiological responses. Likewise, the allelochemicals identified by GC–MS in *O. basilicum* may interfere with physiological and metabolic processes, leading to reduced germination and seedling growth.

Differential inhibitory effects among plant organs can be explained by differences in amount of allelochemical production and release. Guzman-Perez et al (1988) reported that living roots, vegetative tissues and reproductive structures give off a variety of phytotoxic compounds of varying concentrations and biological activity: phenolic compounds, sesquiterpenes, and sesquiterpene lactones. They affect multiple physiological and biochemical processes of seed germination and early seedling development.

The present results also confirm those of Baličević et al. (2015) who showed that watercress seedlings germinated and developed slower under laboratory conditions when they were exposed to aqueous extracts of *O. basilicum* (13.8–27% reduction of germination). It is thought that the inhibitory activity is due to the release of allelochemicals from the decomposing residues of basil as suggested by Chou and Patrick (1976). The allelopathic reactions of maize, squash, tomato, and some agroforestry species after exposure to plant residues or extracts have been reported (Mersie and Singh, 1988; Guzman-Perez et al., 1988; Swaminathan et al., 1990; Evans, 1997; Tamado et al., 2002). Inhibitory effect observed in the present study were concentration-dependent. At the highest concentration (30 g) of both leaf and seed extracts, the highest suppression of germination and seedling growth was observed and comparatively less suppression was observed at the lowest concentration (10 g). Leaf extracts always proved more effective than seed extracts at the same concentration, suggesting that the leaves contain more allelochemicals or higher concentrations. There is also a concentration-dependent inhibitory effect reported by Creamer et al. (1996) who found that the incorporation of

allelopathic barley residues into the soil reduced growth of Setaria glauca by about 81% and Solanum ptychanthum by about 98%. In a similar manner, Purohit and Pandya (2013) reported that basil has strong inhibitory effect on germination and growth of members of Poaceae family. The phytotoxic properties of *O. basilicum* extracts were also confirmed through greenhouse experiments, which showed that with the increase of extract concentration, the percentage of germination decreased, the height of the plants decreased and the accumulation of biomass was reduced compared to the control treatment. In general, inhibition was greater under laboratory conditions than under greenhouse conditions, but was detectable under greenhouse conditions. Such a difference may be due to the adsorption, degradation by microorganisms, dilution and transformation of allelochemicals in the soil environment that decreases their bioavailability and phytotoxicity. The same observations have been made by Xuan et al. (2005), Koodkaew (2017), and Allemann and Denner (2006) and they concluded that allelopathic effects are usually higher under controlled laboratory conditions as compared to soil-based pot or field experiments, where allelochemicals are present in water but not in direct contact with the plant. A separate study of aqueous root exudate extracts likewise reported inhibitory effects on seed germination and seedling growth (Qiu et al., 2025).

Conclusion

It is concluded that aqueous extracts and powders of *O. basilicum* leaves and seeds significantly inhibited the germination, seedling growth, and biomass of *P. glaucum* under laboratory and greenhouse conditions. The inhibitory effects increased with increasing extract concentration, and GC–MS analysis identified several allelochemicals that may contribute to the observed phytotoxic activity. These findings demonstrate the allelopathic potential of *O. basilicum* under controlled experimental conditions. However, additional studies under field conditions are required to validate its efficacy, environmental safety, and practical applicability before recommending its use for weed management.

The present study demonstrated that aqueous extracts and powders of *O. basilicum* leaves and seeds significantly inhibited the germination, seedling growth, and biomass of *P. glaucum* under laboratory and greenhouse conditions. The inhibitory effects increased with extract concentration, and GC–MS analysis identified several bioactive compounds that may contribute to the observed allelopathic activity. These findings suggest that *O. basilicum* possesses promising allelopathic potential under controlled experimental conditions. However, further studies are required to evaluate its effectiveness, persistence,

environmental safety, and practical applicability under field conditions before considering its use as a bioherbicidal agent in sustainable weed management.

The present study demonstrated that the extract from leaves and seeds of *O. basilicum* negatively impacted germination, seedling growth and biomass of *P. glaucum* in both experiments. However, the degree of exposure towards increased levels of aqueous extract concentration varied among all treatments (i-e., 10 g, 20 g and 30 g). Compared to Radicle length, plumule length was more sensitive to the extract from leaves and seeds of *O. basilicum's* aqueous extract. Incorporated of leaves and seeds powder into the soil also inhibited the seedling growth of tested species. GCMS results revealed important allelochemical from the extract of its leaves and seeds and justified the allelopathic property of it. The results demonstrate that *O. basilicum* possesses promising allelopathic potential under controlled laboratory and greenhouse conditions. However, further field-based studies are required to validate its efficacy, environmental safety, and practical applicability before it can be recommended as a bioherbicide for weed management.

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