Research Article

Evaluation of Allelopathic Effects of Leaf Litter Contaminated Soils from Invasive Weeds (*Calotropis procera* and *Paspalum distichum*) on Wheat Germination and Seedling Growth

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Abstract | This experiment has studied the allelopathic effects of two invasive weed species, *Paspalum distichum* L. and *Calotropis procera* L. on wheat (variety Akbar-2019), germination and initial vegetative growth. This was done in soil-filled pots that took place in Gomal University, D.I. Khan, during the Rabi season 2024-25 using a completely randomized five replicates design. The concentration levels of 25g, 50g, 75g, and 100g of the leaf litter per 500g of soil were used to determine their effect as compared to a control treatment. The findings proved that both weeds had a high level of phytotoxic behavior, with the greatest inhibitory ability seen in C. *procera*. Precisely, the 75g and 100g concentrations of C. *procera* completely inhibited the wheat germination (0 %) and seedling development, giving it a germination index of (0.00). At concentration of 50g, the germination and the length of the seedling were significantly reduced by the individual to (25 %) in comparison with the control. By contrast, P. distichum had relatively milder effects on wheat growth parameters, including germination (%) mean germination time, seedling length and crop growth rate, However, maximum germination (62.50%), mean germination time (4.18 days), chlorophyll content, (29.98 SPAD) seedling length, (23.01 cm) and crop growth rate, (5.92 mg m^{-2} per day^{-1}) was observed in control (where no leaf litter application was done). The research concludes therefore that, the establishment of wheat by the presence of the allelopathic agent of C. *procera* is a serious challenge that needs to be controlled to maintain crop yield in wheat plantations.

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Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, serving as a major source of food for humans and a valuable feed resource for livestock (Khan *et al*., 2022). It is the most widely cultivated crop across diverse agro-ecological regions and plays a vital role in global food security. Wheat straw is extensively used for producing by-products that are either incorporated into the soil as organic manure or utilized as animal feed. Wheat is the principal staple food of the Eastern Gangetic Plains (EGP) of South Asia, including Bangladesh, Pakistan, Southern Nepal, and Eastern India (Pokhrel *et al*., 2013). In Pakistan, wheat occupies approximately 9.6 million hectares with an annual production of about 31.4 million tons, highlighting its economic and agronomic importance.

Allelopathy is a biological phenomenon involving the production and release of secondary metabolites by plants that influence the growth and development of surrounding plants and organisms (Khamare *et al*., 2022). In modern agriculture, allelopathy has gained attention as a natural tool for weed suppression, sustainable crop production, soil conservation, pest and disease management, and biodiversity enhancement (An *et al*., 1998). Allelopathic interactions may be either beneficial or detrimental and occur when plants release allelochemicals into the environment through processes such as residue decomposition, root exudation, volatilization, and leaching, thereby affecting nearby plants (Weston, 2005). Several crops, including sorghum (*Sorghum bicolor* L.), alfalfa (*Medicago sativa* L.), black mustard (*Brassica nigra* L.), sunflower (*Helianthus annuus* L.), and buckwheat (*Fagopyrum esculentum* L.), possess allelopathic potential, with allelochemicals distributed in various plant parts such as roots, stems, leaves, flowers, and seeds. These properties also allow certain allelopathic plants to be used as mulches in conservation farming systems (Wu *et al*., 2001).

Calotropis procera (giant milkweed) is a perennial medicinal shrub belonging to the family Asclepiadaceae and is commonly found in arid and semi-arid regions (Radhaboy *et al*., 2019). The species thrives under hot climatic conditions with dry, sandy, and alkaline soils and reproduces mainly through seeds (Orwa *et al*., 2009; Hassan *et al*., 2015). Its invasive success is attributed to strong reproductive capacity and tolerance to harsh environments (Kaur *et al*., 2021). The plant contains various bioactive compounds, including flavonoids, tannins, cardiac glycosides, alkaloids, and triterpenes, which exhibit antioxidant, pharmacological, and growth-inhibitory properties (Radwan *et al*., 2019; Ghramh *et al*., 2021). The continuous presence of *C. procera* near crop fields has been reported to cause adverse effects on crops such as sorghum, maize, cotton, and sugarcane through chemical interference. In D.I. Khan district, *C. procera* is recognized as a major invasive shrub negatively affecting fruits, vegetables, and field crops.

Paspalum distichum L. (knotgrass or joint grass) is a perennial invasive grass species that has caused substantial ecological and agricultural losses worldwide. The species spreads rapidly through stolons and rhizomes and shows high adaptability across diverse climatic zones, including tropical and subtropical regions of America, Europe, Asia, Africa, Australia, and New Zealand. In wetland and aquatic ecosystems, *P. distichum* forms dense mats that obstruct irrigation channels and drainage systems, while its rapid expansion has led to a considerable reduction in native plant species (Ashiq and Zubair, 2014).

Weeds are a persistent constraint in wheat production systems and can release allelochemicals that negatively affect wheat germination and early seedling growth. Such allelopathic effects are particularly harmful in late-sown wheat, resulting in poor crop establishment and reduced productivity. Various weeds through their stubbles and residues may suppress germination and growth of economic crops like wheat by releasing many phyto chemicals directly or after decomposition into the environment (Hussain *et al*., 2025). However, limited information is available on the comparative allelopathic effects of invasive weed leaf litter on wheat germination and early growth (Hussain *et al*., 2026).

Objectives of study

This trial was carried out to investigate phytotoxicity of two weeds on wheat. To determine the allelopathic potential of *Calotropis procera* and *Paspalum distichum* on the wheat growth by altering their leaves litter concentrations.

Materials and Methods

Study site

This experiment was carried out for four consecutive weeks during Rabi season 2024-25 in the laboratory of Department of Agronomy, Gomal University, D.I. Khan.

Experimental design

This study was conducted under laboratory condition by using glasses as experimental pots at ambient temperature. All necessary facilities including well-equipped laboratory for conducting research work, growth measuring instruments, and modern data recording tools, statistical analysis software, yield assessment devices, computational facilities, and technical staff were available during the study period. This study was executed in a completely randomized design with 5 replications. Leachate of *Calotropis procera* L. and *Paspalum distichum* L. was mixed in the soil. Wheat variety (Akbar-2019) was used in this experiment and its seed was obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan.

Experimental soil characterized

The experimental soil was characterized by a pH of 8.4 and a sodium adsorption ratio (SAR) of 11.4. The electrical conductivity (EC) was measured at 1.74 dS m^{-1}. Textural analysis revealed a composition of 36.39% sand, 24.79% silt, and 45.7% clay, classifying the soil texture as clay loam. Additionally, the soil was found to have an organic matter content of 0.42%.

Sample collection

Fresh and healthy leaves of giant milkweed (*Calotropis procera* L.) and knotgrass (*Paspalum distichum* L.) were collected from the experimental area of Gomal University, D.I. Khan. After collection, the leaves were cleaned with distilled water and allowed to air dry at ambient temperature in a dark area to prevent any unfavorable damage from sunlight. The dried leaves were crushed with hands and converted into powder form using electric blender. Leachate of both invasive weed species as mentioned above was mixed (@ 25g, 50g, 75g, and 100g) in 500g of soil. After incorporating, the soil was irrigated with tap water and was left bare so that the leaf powder of both weeds starts to decompose and release allelochemicals.

Treatments detail

The experiment consisted of nine treatments: T1 served as the control with no leaf litter application; T2 received 25g of *Paspalum distichum* leaf litter; T3 received 50g of *Paspalum distichum* leaf litter; T4 received 75g of *Paspalum distichum* leaf litter; T5 received 100g of *Paspalum distichum* leaf litter; T6 received 25g of *Calotropis procera* leaf litter; T7 received 50g of *Calotropis procera* leaf litter; T8 received 75g of *Calotropis procera* leaf litter; and T9 received 100g of *Calotropis procera* leaf litter.

Observations

Germination and emergence metrics

Seed germination (%): Calculated by counting the number of seeds that sprouted and dividing it by the total number of seeds sown, then multiplying by 100.
Germination index (GI): This is calculated by counting the number of germinated seeds daily. It uses a formula where the number of germinated seeds is divided by the day of the count and summed up.
Mean germination time (MGT): Measured in days. It identifies how long it takes, on average, for a seed to germinate. Lower MGT usually means higher seed vigor.
Germination count (Number): A simple daily physical count of seeds that have successfully broken the seed coat and showed a radical or plumule.
Final emergence (%): Usually recorded in soil based studies. It is the percentage of seedlings that actually push through the soil surface and become visible.
Morphological growth (Length): Measured using a standard ruler or measuring tape.
Seedling length (cm): The total length of the plant from the very tip of the root to the highest tip of the shoot.
Root and shoot length (cm): These are measured separately. Root length starts from the base of the seed to the longest root tip shoot length starts from the seed to the highest leaf tip.
Specific seedling length: This is a ratio, calculated by dividing the total seedling length by the seedling dry weight.

Physiological and biomass metrics

These require specific equipment like SPAD meters, electric balances, and drying ovens.
Chlorophyll content (SPAD value): Measured using a handheld SPAD 502 meter. You clip the device onto a leaf, and it provides a non destructive reading of the greenness (chlorophyll density).
Fresh root/shoot weight (mg): The roots and shoots are separated and weighed on a high-precision digital scale immediately after harvesting to prevent moisture loss.
Dry root/shoot weight (mg): The samples are placed in a paper bag and dried in an oven (usually at 70 °C

48 to 72 hours) until the weight becomes constant. This removes all water.

Calculated growth rate

Crop growth rate (mg m^{-2} day^{-1}): This measures how much "mass" the crop adds over a specific land area per day.

$$\text{Formula: CGR} = \frac{W2 - W1}{T2 - T1}$$

Where; *W* is weight, *A* is ground area, and *T* is time in days.

Statistical analysis

Data was statistically analyzed using Statistix 10.1 software through Analysis of Variance (ANOVA) technique. Mean comparisons among treatments were performed using the LSD test at a 5% significance level, following the guidelines of (Steel *et al*., 1997).

Results

Germination (%)

Germination of seed provides the basis for crop development and economical yield. The results indicated that use of 75g and 100g of *Calotropis procera* L. completely inhibited the germination of wheat compared to use of 75g and 100g of *Paspalum distichum* L. (Figure 1). However, the highest germination (62.5%) was noted where no leaf litter was used which was statistically similar with *Paspalum distichum* L. @ 25g application. Whereas, the lowest germination (25%) was observed in *Calotropis procera* @ 50g respectively. Plants' allelopathic effect relies on the direct release of allelochemicals into the soil through decomposition.

Germination index (%)

Germination index of wheat under leaves litter of two weeds are presented in (Figure 1) Germination index is a general ability of a crop that showed activity of the embryo towards emergence and seedling development. It is a test to determine viable seed count in a particular lot for the beginning of crop growth. The results showed that applying 75g and 100g of *Calotropis procera* L. completely inhibited germination index of wheat, in contrast to the same concentration of *Paspalum distichum* L. which did not produce such drastic effects. The maximum germination index (0.14%), was noted in the control, where no leaf litter was applied, which was statistically similar with the application of 25g of *Paspalum distichum* L. On the other hand, the minimum germination index (0.10%) was observed in the case of *Calotropis procera* L. @ 50g. The decreased germination in wheat seedlings can be directly linked to the suppressing effects of allelochemicals found in these invasive weed species. These natural chemical compounds interfere with integral cellular processes, including cell division and various physiological functions within the target plant seedlings.

Mean germination time (days)

The mean germination time represents the average duration, measured in days that seeds require to complete their germination process and physiology. A lower mean germination time (MGT) value is indicative of a faster germination rate, which is generally more desirable in agricultural applications. The results showed (Figure 1) that the use of 75g and 100g of *Calotropis procera* L. completely suppressed MGT of wheat. The minimum MGT (4.18 days) was recorded with the control treatment, which was statistically similar with all other treatments.

Germination count (Number)

Germination count refers to the process of evaluating the number of seeds in a given sample that have successfully sprouted and begun developing into seedlings. This measurement serves as an important indicator of seed vigor and viability. The results indicated that no germination count was recorded with application of 75g and 100g of *Calotropis procera* L. compared with the use of 75g and 100g of *Paspalum distichum* L. In the control treatment, (Figure 1) wheat seeds exhibited a maximum germination count which was statistically similar with application of *Paspalum distichum* at 25g concentration. *Paspalum distichum* at 25g concentration. While the minimum germination count (1.90) was recorded with *Calotropis procera* @ 50g.

Final emergence (%)

Seedling emergence represents the most crucial factor determining the success of yearly crops. Uniform and quick seedling emergence maximizes yield potential by optimizing canopy structure, reducing competition, and enhancing weed control (Figure 1). The results indicated that application of 75g and 100g of *Calotropis procera* L. completely inhibited final emergence of wheat, compared with

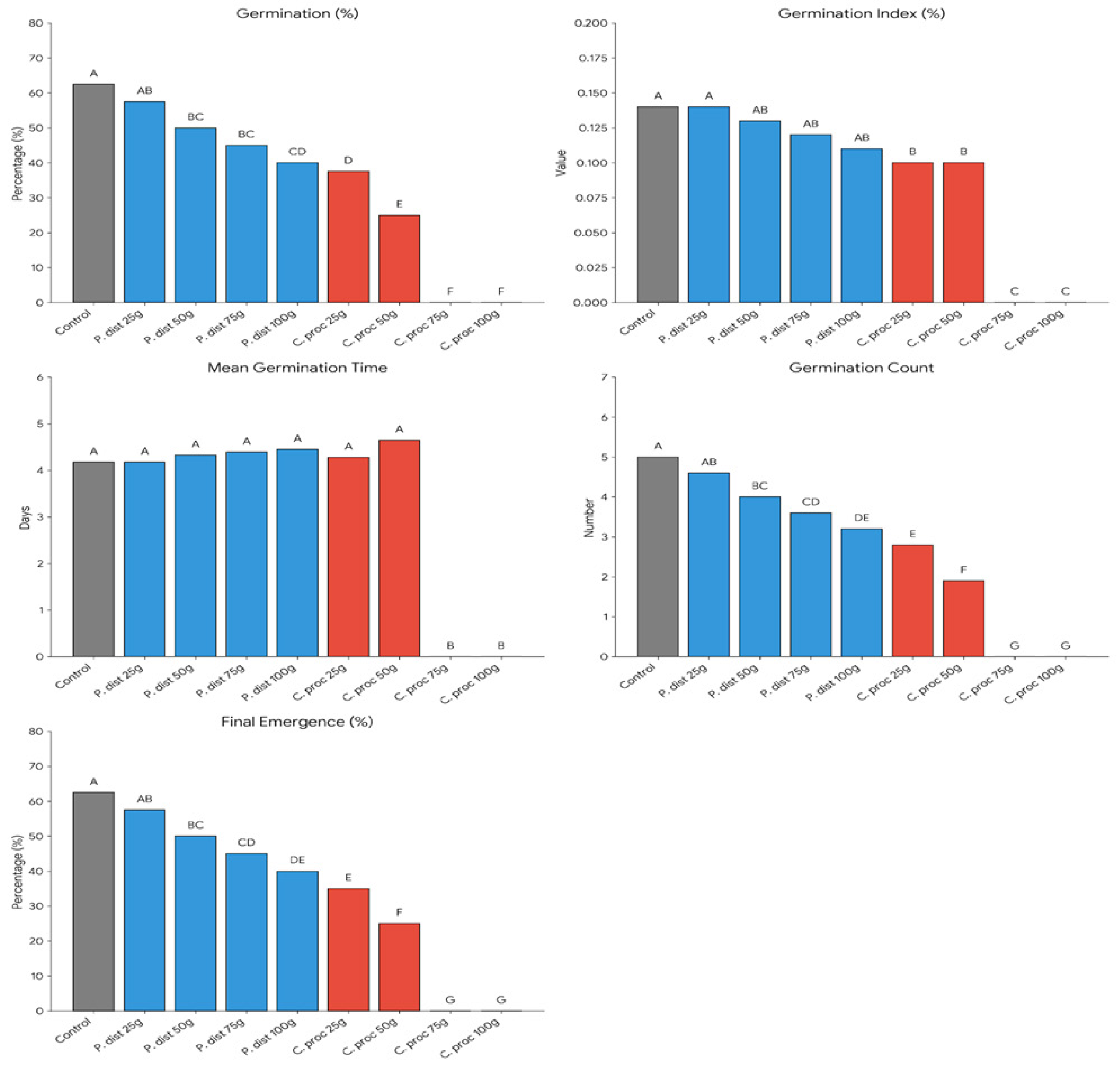

Figure 1: *Allelopathic influence of soil-incorporated leaf litter of Paspalum distichum L. and Calotropis procera on the germination dynamics and emergence of wheat (Triticum aestivum L.).*

the same concentrations of *Paspalum distichum* L. The maximum final emergence (62.50%) was noted in control treatment where no weed leaf litter was used which was statistically similar with application of 25g *Paspalum distichum* (57.50%). While the minimum final emergence (25.00%) was noted with application of *Calotropis procera* at 50g, followed by (35.00%) with the use of *Calotropis procera* at 25g. As discussed above, *Calotropis procera* reduced both the germination percentage and germination index of wheat, which in turn negatively affected final emergence. This reduction can be attributed to the release of phytotoxic compounds during residue decomposition.

Chlorophyll content (SPAD value)

Chlorophyll is the primary photosynthetic pigment in green plants and is essential for light absorption and energy conversion. The results indicated that application of *Calotropis procera* L. at 75 g and 100 g significantly reduced the chlorophyll content of wheat at both 14 and 28 days after sowing. In contrast, similar concentrations of *Paspalum distichum* L. did not show any inhibitory effect on chlorophyll content (Figure 2). At 14 days after sowing, the maximum chlorophyll content (20.38) was recorded in the control treatment, which was statistically similar to *P. distichum* at 25 g, while the minimum value (9.67) was observed with *C.*

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procera at 50 g. At 28 days after sowing, the highest chlorophyll content (29.98) was recorded in the control treatment, whereas the lowest value (9.57) was noted with *C. procera* at 50 g. Moreover, the greatest increase in chlorophyll content between 14 and 28 days after sowing occurred in the control treatment, indicating normal plant growth and chlorophyll synthesis.

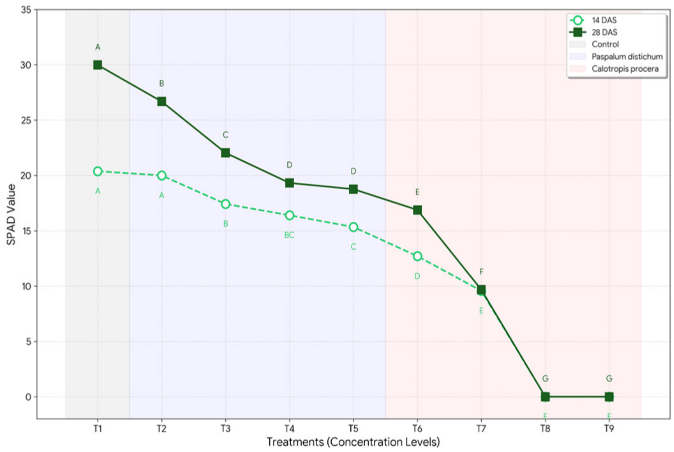

Figure 2: *Periodic assessment of chlorophyll content (SPAD values) in wheat (Triticum aestivum L.) as influenced by soil-incorporated leaf litter of Paspalum distichum and Calotropis procera at 14 and 28 days after sowing (DAS).*

Seedling length (cm)

Seedling length of wheat was significantly affected by the application of leaf litter from both invasive weed species (Table 1). Application of *Calotropis procera* L. at 75 g and 100 g completely inhibited seedling growth compared with the corresponding concentrations of *Paspalum distichum* L. At 14 days after sowing (DAS), the maximum seedling length (16.82 cm) was recorded in the control treatment, which was statistically similar to *P. distichum* at 25 g and 50 g, whereas the minimum value (8.20 cm) was observed with *C. procera* at 50 g. At 28 DAS, the highest seedling length (23.01 cm) was recorded in the control treatment, followed by *P. distichum* at 25 g, while the lowest seedling length (9.20 cm) was noted with *C. procera* at 50 g. The greatest increase in seedling length between 14 and 28 DAS (6.19 cm) was observed in the control treatment. The reduction in seedling length under *C. procera* treatments may be attributed to the release of phytotoxic allelochemicals that impair photosynthetic efficiency and biomass accumulation in wheat seedlings.

Root length (cm)

Root length of wheat was significantly influenced by the application of leaf litter from the invasive weed species (Table 2). Application of *Calotropis procera* L. at 75 g and 100 g markedly suppressed radicle development compared with the corresponding concentrations of *Paspalum distichum* L. At 14 days after sowing (DAS), the maximum root length (6.92 cm) was recorded in the control treatment, followed by *P. distichum* at 25 g (6.42 cm), whereas the minimum root length (2.26 cm) was observed with *C. procera* at 50 g. Similarly, at 28 DAS, the highest root length

Table 1: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the seedling length of wheat.*

S. No	Treatments	Seedling length (cm) 14 DAS	Seedling length (cm) 28 DAS	Difference in length (cm)
T_1	Control	16.82 a	23.01 a	6.19 a
T_2	*Paspalum distichum* 25g	16.04 ab	20.30 b	4.26 b
T_3	*Paspalum distichum* 50g	16.00 ab	20.22 b	4.22 b
T_4	*Paspalum distichum* 75g	15.64 b	19.08 c	3.44 b
T_5	*Paspalum distichum* 100g	15.58 b	19.28 c	3.70 b
T_6	*Calotropis procera* 25g	9.80 c	13.74 d	3.94 b
T_7	*Calotropis procera* 50g	8.20 d	9.40 e	1.20 c
T_8	*Calotropis procera* 75g	0.00 e	0.00 f	0.00 d
T_9	*Calotropis procera* 100g	0.00 e	0.00 f	0.00 d
$LSD_{0.05}$		0.92	0.84	0.97

DAS = Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

Table 2: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the root length of wheat.*

S. No	Treatments	Root length (cm) 14 DAS	Root length (cm) 28 DAS	Difference in Root length (cm)
T_1	Control	6.92 a	11.92 a	5.00 a
T_2	*Paspalum distichum* 25g	6.42 b	11.10 b	4.68 b
T_3	*Paspalum distichum* 50g	6.40 bc	10.96 b	4.56 bc
T_4	*Paspalum distichum* 75g	6.08 bc	10.20 c	4.12 c
T_5	*Paspalum distichum* 100g	5.96 c	10.06 c	4.10 c
T_6	*Calotropis procera* 25g	4.18 d	7.26 d	3.08 d
T_7	*Calotropis procera* 50g	2.26 e	3.51 e	1.25 e
T_8	*Calotropis procera* 75g	0.00 f	0.00 f	0.00 f
T_9	*Calotropis procera* 100g	0.00 f	0.00 f	0.00 f
$LSD_{0.05}$		0.45	0.47	0.37

DAS= Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

(11.92 cm) was recorded in the control treatment, followed by *P. distichum* at 25 g, while the lowest value (3.51 cm) was noted with *C. procera* at 50 g. The greatest increase in root length between 14 and 28 DAS was observed in the control treatment, indicating normal root development in the absence of weed leaf litter.

Fresh root weight (mg)

Fresh root weight was significantly affected by the application of leaf litter from the two invasive weed species (Table 3). Application of *Calotropis procera* L. at 75 g and 100 g completely inhibited root initiation and fresh root weight compared with the same concentrations of *Paspalum distichum* L. At 14 days after sowing (DAS), the highest fresh root weight (92.00 mg) was recorded in the control treatment, while the lowest value (28.00 mg) was observed with *C. procera* at 50 g. At 28 DAS, the maximum fresh root weight (144.00 mg) was recorded in the control treatment, followed by *P. distichum* at 25 g (122.00 mg), whereas the minimum value (46.00 mg) was noted with *C. procera* at 50 g. The greatest increase in fresh root weight between 14 and 28 DAS was recorded in the control treatment. The reduction in fresh root weight under *C. procera* treatments may be attributed to allelochemicals released during decomposition, which can limit root growth and water uptake (Zohaib *et al*., 2016).

Table 3: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the fresh root weight of wheat.*

S. No	Treatments	Fresh root weight (mg) 14 DAS	Fresh root weight (mg) 28 DAS	Difference in weight
T_1	Control	92.00 a	144.00 a	52.00 a
T_2	*Paspalum distichum* 25g	78.00 b	122.00 b	44.00ab
T_3	*Paspalum distichum* 50g	76.00 b	114.00 bc	38.00bc
T_4	*Paspalum distichum* 75g	68.00 b	102.00 c	34.00bc
T_5	*Paspalum distichum* 100g	57.00 c	89.00 d	32.00 c
T_6	*Calotropis procera* 25g	39.00 d	69.00 e	30.00 c
T_7	*Calotropis procera* 50g	28.00 d	46.00 f	18.00 d
T_8	*Calotropis procera* 75g	0.00 e	0.00 g	0.00 e
T_9	*Calotropis procera* 100g	0.00 e	0.00 g	0.00 e
$LSD_{0.05}$		10.81	12.89	11.47

DAS = Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

Fresh shoot weight (mg)

Fresh shoot weight of wheat was significantly reduced by the application of *Calotropis procera* L. at 75 g and 100 g at both 14 and 28 days after sowing (Table 4). At 14 DAS, the maximum fresh shoot weight (112.00 mg) was recorded in the control treatment, followed by *Paspalum distichum* at 25 g (98.00 mg), while the minimum value (30.00 mg) was observed with *C. procera* at 50 g. Similarly, at 28 DAS, the highest fresh shoot weight (174.00 mg) was recorded in the control treatment, whereas the lowest value (50.00 mg) was noted with *C. procera* at 50 g. The greatest increase in fresh shoot weight between 14 and 28 DAS was recorded in the control treatment. The reduction in shoot weight under weed treatments may be attributed to allelochemicals that impair nutrient and water uptake, thereby limiting plant growth.

Table 4: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the fresh shoot weight of wheat.*

S. No	Treatments	Fresh shoot weight (mg) 14 DAS	Fresh shoot weight (mg) 28 DAS	Difference in weight
T_1	Control	112.00 a	174.00 a	62.00 a
T_2	*Paspalum distichum* 25g	98.00 b	138.00 b	40.00 b
T_3	*Paspalum distichum* 50g	90.00 bc	128.00bc	38.00 b
T_4	*Paspalum distichum* 75g	86.00 bc	112.00 c	26.00 b
T_5	*Paspalum distichum* 100g	82.00 c	106.00 c	24.00 b
T_6	*Calotropis procera* 25g	58.00 d	82.00 d	24.00 b
T_7	*Calotropis procera* 50g	30.00 e	50.00 e	20.00 c
T_8	*Calotropis procera* 75g	0.00 f	0.00 f	0.00 d
T_9	*Calotropis procera* 100g	0.00 f	0.00 f	0.00 d
$LSD_{0.05}$		13.24	17.98	9.84

DAS= Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

Dry root weight (mg)

Dry root weight was significantly reduced by the application of *Calotropis procera* L. at 75 g and 100 g, which completely inhibited root initiation and development compared with the corresponding concentrations of *Paspalum distichum* L. (Table 5). At 14 days after sowing (DAS), the highest dry root weight (48.00 mg) was recorded in the control treatment, while the lowest value (8.00 mg) was observed with *C. procera* at 50 g, which was statistically similar to *C. procera* at 25 g. At 28 DAS, the maximum dry root weight (84.00 mg) was recorded in the control treatment, followed by *P. distichum* at 25 g (64.00 mg), whereas the minimum value (13.00 mg) was noted with *C. procera* at 50 g. The greatest increase in dry root weight between 14 and 28 DAS was observed in the control treatment. The reduction in dry root weight under weed treatments may be attributed to the phytotoxic effects of allelochemicals released from the invasive species.

Table 5: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the dry root weight of wheat.*

S. No	Treatments	Dry root weight (mg) 14 DAS	Dry root weight (mg) 28 days	Difference in weight (mg)
T_1	Control	48.00 a	84.00 a	36.00 a
T_2	*Paspalum distichum* 25g	34.00 b	69.00 b	35.00 a
T_3	*Paspalum distichum* 50g	28.00 bc	60.00 b	32.00 ab
T_4	*Paspalum distichum* 75g	26.00 bc	54.00 b	28.00 b
T_5	*Paspalum distichum* 100g	22.00 cd	40.00 c	18.00 c
T_6	*Calotropis procera* 25g	14.00 de	28.00 d	14.00 cd
T_7	*Calotropis procera* 50g	8.00 e	13.00 e	5.00 d
T_8	*Calotropis procera* 75g	0.00 f	0.00 e	0.00 e
T_9	*Calotropis procera* 100g	0.00 f	0.00 e	0.00 e
$LSD_{0.05}$		8.55	10.38	7.78

DAS = Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

Table 6: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the dry shoot weight of wheat.*

S. No	Treatments	Dry shoot weight (mg) 14 DAS	Dry shoot weight (mg) 28 DAS	Difference in weight (mg)
T_1	Control	58.00 a	102.00 a	44.00 a
T_2	*Paspalum distichum* 25g	46.00 b	82.00 b	36.00 ab
T_3	*Paspalum distichum* 50g	40.00 bc	76.00 bc	36.00 ab
T_4	*Paspalum distichum* 75g	40.00 bc	72.00 c	32.00 b
T_5	*Paspalum distichum* 100g	34.00 c	62.00 c	28.00 b
T_6	*Calotropis procera* 25g	16.00 d	36.00 d	20.00 c
T_7	*Calotropis procera* 50g	10.00 de	23.00 e	13.00 c
T_8	*Calotropis procera* 75g	0.00 e	0.00 f	0.00 d
T_9	*Calotropis procera* 100g	0.00 e	0.00 f	0.00 d
$LSD_{0.05}$		10.56	10.73	8.65

DAS = Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

Dry shoot weight (mg)

Dry shoot weight represents the mass of a plant's above-ground parts after all moisture has been removed. It serves as an indicator of the dry matter or biomass of the shoot system. The results indicated that application of 75g and 100g of *Calotropis procera* L. completely inhibited the dry shoot weight of wheat compared with the use of 75g and 100g of *Paspalum distichum* L. (Table 6). The maximum dry shoot weight (58.00 mg), after 14 days of sowing was recorded in the control treatment, followed by (46.00 mg), with *Paspalum distichum* at 25g, While the minimum dry shoot weight (10.00 mg), was recorded with *Calotropis procera* at 50g, respectively. However, the maximum dry shoot weight (102.00 mg), 28 DAS was recorded in the control treatment, whereas the minimum dry shoot weight (23.00 mg), was recorded with *Calotropis procera* @ 50g. The maximum increase in dry shoot weight between 14 and 28 DAS was with control treatment where no leaf litter was applied in soil.

Table 7: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on specific seedling length of wheat.*

S. No	Treatments	Specific seedling length 14 DAS	Specific seedling length 28 DAS	Difference in length
T_1	Control	0.41 a	0.85 a	0.44 a
T_2	*Paspalum distichum* 25g	0.40 a	0.68 a	0.28 b
T_3	*Paspalum distichum* 50g	0.29 b	0.49 b	0.20 bc
T_4	*Paspalum distichum* 75g	0.27 b	0.43 bc	0.16 bcd
T_5	*Paspalum distichum* 100g	0.27 b	0.41 bc	0.14 cd
T_6	*Calotropis procera* 25g	0.24 b	0.35 bc	0.11 cd
T_7	*Calotropis procera* 50g	0.22 b	0.29 c	0.07 de
T_8	*Calotropis procera* 75g	0.00 d	0.00 c	0.00 e
T_9	*Calotropis procera* 100g	0.00 d	0.00 c	0.00 e
$LSD_{0.05}$		0.07	0.14	0.10

DAS= Days after sowing; Means followed by the same letter are not significantly different at $p \leq 0.05$

Specific seedling length

Specific seedling length, which represents the mathematical relationship between a seedling length and its dry weight, carefully measures its growth process. This ratio serves as a crucial indicator for monitoring and evaluating seedling development. The findings indicated that use of 75g and 100g of *Calotropis procera* L. completely hindered the specific seedling length of wheat compared with the use of 75g and 100g of *Paspalum distichum* L. (Table 7). At 14 DAS the maximum specific seedling length (0.41), was noted with control, which was statistically similar with *Paspalum distichum* @ 25g, While the minimum specific seedling length (0.22), was noted with *Calotropis procera* @ 50g respectively. In contrast, the highest specific seedling length 28 DAS (0.85), was recorded with control treatment which was statistically similar with *Paspalum distichum* @ 25g, While, the lowest specific seedling length (0.29), was recorded with *Calotropis procera* @ 50g, respectively. Similarly, the maximum increase in specific seedling length between 14 and 28 DAS was noted with control treatment.

Crop growth rate (mg m^{-2} day^{-1})

Crop growth rate quantifies the rate at which a crop accumulates dry matter per unit area over a specific period. It reflects how efficiently a crop gains biomass within a defined area and timeframe. The results showed that the application of 75g and 100g of *Calotropis procera* L. completely inhibited crop growth rate compared to the same concentrations of *Paspalum distichum* L. (Table 8). In the control treatment, wheat seeds showed the highest growth rate (5.92 mg), which was statistically similar with *Paspalum distichum* at 25g. While the lowest crop growth rate was recorded (0.92 mg) with *Calotropis procera* at 50g. These findings aligned closely with the research outcomes of that *Calotropis procera* aqueous leaf extract has allelopathic effect on growth rate of wheat. The leaf, stem, and root extracts reduced crop growth and net assimilation rates, with leaf extract at 40% concentration showing the strongest inhibitory effect.

Table 8: *Influence of soil incorporation of Paspalum distichum L. and Calotropis procera L. leaves litter on the crop growth rate of wheat.*

S. No	Treatments	Crop growth rate (mg m^{-2} day^{-1})
T_1	Control	5.92 a
T_2	*Paspalum distichum* 25g	5.14 ab
T_3	*Paspalum distichum* 50g	4.71b
T_4	*Paspalum distichum* 75g	4.08 bc
T_5	*Paspalum distichum* 100g	3.57 c
T_6	*Calotropis procera* 25g	2.42 d
T_7	*Calotropis procera* 50g	0.92 e
T_8	*Calotropis procera* 75g	0.00 f
T_9	*Calotropis procera* 100g	0.00 f
$LSD_{0.05}$		0.88

Means followed by the same letter are not significantly different at $p \leq 0.05$

Discussion

Hussain *et al.* (2019) reported that allelochemicals released by plant parts such as leaves had negative effect on the seed germination. Moreover, this inhibitory effect becomes more pronounced with the higher concentration of allelopathic materials. These allelochemicals may penetrate the seed coat, disrupting membrane integrity and causing leakage of cellular contents, thereby compromising embryo viability. Furthermore, they can inhibit water imbibition by altering osmotic potential around the seed, resulting in delayed or failed activation of metabolic pathways essential for germination (Singh *et al.*, 2009). In crux, application of *Calotropis procera* at concentrations of 75 g and above likely damaged or killed the seed embryo, resulting in complete inhibition of germination.

These results are in correspondence to the study of (Muhammad and Majeed, 2014), who found that use of aqueous extract of sunflower remarkably reduced the germination rate and germination index of wheat. Furthermore, dry water extracts led to slightly lower germination rates and decreased germination index compared to control.

The results of this study aligned with the findings of (Naeem *et al.*, 2023), who studied the effects of cotton plant extracts on seed development and early growth of wheat and found that seeds treated with crude extract of stem showed significant increase in MGT compared to the control group. Similar results were reported by (Sampietro *et al.*, 2006), that phenolic compounds cause delay in seed germination by disrupting seed dormancy and activities of various enzymes. The delay was attributed to the presence of allelochemicals, which affected cell division and energy metabolism in the germinating embryo. Kaur *et al.* (2012) observed increased MGT in wheat seeds treated with *Ageratum conyzoides* extract, noting that the allelopathic impact was dose-dependent. Higher concentrations of the extract led to more pronounced delays in germination time, likely due to oxidative stress induced by phenolic compounds.

Our results are in agreement with (Samreen *et al.*, 2009), who reported that *Calotropis procera* exerts strong allelopathic effects, likely due to volatile phenolic compounds that can inhibit seed sprouting by suppressing the synthesis of indole acetic acid and germination-related enzymes. Similarly, (Mushtaq *et al.*, 2025), found that aqueous leaf extracts of *C. procera* significantly reduced seed germination, germination count, and subsequent plant growth. This inhibition is primarily attributed to water-soluble organic acids in the leaf extracts, which interfere with normal germination processes. Dolianitis et al. (2005), further confirmed that elevated concentrations of *C. procera* allelochemicals suppress germination by blocking the production of indole acetic acid and other essential enzymes.

The presence of water-soluble allelochemicals in *C. procera* leaf extracts has been shown to significantly delay seed germination, suppress final emergence, and impair subsequent crop growth parameters (Turk and Tawaha, 2003).

These findings are consistent with (Khan *et al.*, 2007), who reported that various weed species exert strong allelopathic effects on chlorophyll content, likely through disruption of metabolic processes or direct damage to chloroplasts responsible for chlorophyll synthesis. Comparable results were obtained by Vaithiyanathan et al. (2014), who observed significant reductions in chlorophyll levels in different plants when treated with tobacco and neem extracts.

Hussain *et al.* (2024) showed a comparable drop in seedling moisture content due to phytotoxic effects. This decline shows that hazardous chemicals in the extract interfere with cell division, resulting in a reduction in root cell proliferation. This may cause a decrease in mineral uptake, nutritional absorption, and nutrient transfer from the root to other plant components resulted in decline in growth.

The inhibitory effect of allelochemicals on wheat root length can be attributed to several interrelated physiological and biochemical disruptions, with reduced cell division being a primary factor. Allelochemicals can impair meristematic activity at the root tips, slowing the production of new cells and thereby limiting elongation. Gholami et al. (2011) noted that one of the earliest visible effects of these compounds is cellular disruption, often resulting from damage to the plasma membrane. This membrane injury alters permeability, causing leakage of vital solutes and disrupting ion gradients essential for cell expansion. In addition to physical damage, allelochemicals may interfere with DNA replication and mitotic processes, further suppressing root meristem activity. They can also inhibit the synthesis of essential hormones such as auxins and cytokinins, which regulate root initiation and elongation. Disruption of auxin transport, in particular, can severely limit the formation of lateral and primary roots.

These results are in agreement with the findings of (Hussain *et al.*, 2022), who discovered that applying an aqueous extract of *P. deltoides* considerably reduced the root biomass of different crops. Khan *et al.* (2007), investigated the allelopathic effect of different concentrations of *Calotropis procera* leaves, stems, and roots in aqueous extracts on wheat crops and confirmed that the *Calotropis procera* should be removed from areas adjacent to wheat fields because it releases certain allelopathic substances that seriously harm the crop.

Our results are consistent with (Khan et al., 2008), who reported decreased fresh weight in maize seedlings following exposure to aqueous extracts of *Prosopis juliflora*. Similarly, (Shah et al., 2018) found that allelochemicals from *C. lancifolius* extracts lowered the fresh weight of maize by reducing photosynthetic capacity. This reduction indicates that allelochemicals interfere with cell division, limiting root cell proliferation, which in turn decreases mineral uptake, nutrient absorption, and translocation to aerial parts ultimately resulting in reduced biomass accumulation.

These findings align with (Xaxa *et al.*, 2018), who reported that *P. deltoides* leaf litter reduced dry biomass in bread wheat and suppressed photosynthesis, ultimately decreasing wheat productivity in areas sheltered by *P. deltoides*.

Our findings are consistent with (Singh *et al.*, 2010), who attributed the reduction in dry shoot biomass to the phytotoxic effects of *C. procera* extract. Similarly, their study reported that *eucalyptus* extracts decreased the dry shoot biomass of *Zea mays*. Gulzar *et al.* (2014) further demonstrated that several allelochemicals inhibit root cell absorption of macro- and micronutrients and enhance IAA oxidase activity, collectively leading to significant reductions in both fresh and dry biomass.

Previous studies have shown that during the early growth stage, fewer allelochemicals are released due to the partial decomposition of plant residues, resulting in minimal positive or negative effects on crop growth. Over time, as decomposition progresses, the release of allelochemicals increases, leading to stronger growth inhibition in wheat (Zhang *et al*., 2024).

The presence of allelochemicals, such as phenolics and cardenolides, significantly impairs seedling establishment by disrupting metabolic pathways. These compounds inhibit key enzymatic activities including α-amylase, SOD, POD, and CAT which are essential for carbohydrate breakdown and antioxidant defense. Consequently, this metabolic interference reduces germination vigor, germination index (%), and germination count, while extending the mean germination time (days). As physiological resources are diverted from biomass accumulation to stress mitigation, overall crop growth rates (mg m^{-2} day^{-1}) decline. Recent research by Hussain *et al*. (2025) emphasizes that management approaches involving herbicides or allelopathic extracts can profoundly influence the growth and yield of crops like maize (*Zea mays* L.). Their findings align with the observations of (Valthiyanathan *et al*., 2014) and (Manzoor *et al*., 2013), confirming that phytotoxic effects are dose-dependent. At higher concentrations, these inhibitors significantly reduce physical parameters, including final emergence (%), chlorophyll content (SPAD value), seedling and root lengths (cm), and both the fresh and dry weights (mg) of roots and shoots. Ultimately, such stress results in lower specific seedling length, reflecting a systemic failure in resource allocation during early growth.

Allelochemicals like phenolics and cardenolides impair key enzymes including α-amylase, SOD, POD, and CAT disrupting carbohydrate breakdown, antioxidant defense, and stress mitigation during early growth. This metabolic interference lowers germination vigor and slows growth rate, as physiological resources are diverted to stress responses rather than biomass accumulation. Valthiyanathan *et al*. (2014) confirmed that phytotoxic effects on plants directly correlate with concentration level the effect becomes more severe as dosage increases. Similar findings were documented by Manzoor *et al*. (2013), who found that *Calotropis procera* leaf extract in water demonstrated inhibitory effects on lentil growth, with the highest concentration significantly decreasing all measured physical growth parameters.

Conclusion

Soil incorporation of leaf litter of *Calotropis procera* and *Paspalum distichum* exert allelopathic effect on wheat growth, with *Calotropis procera* exhibiting a strong allelopathic effect at higher concentrations (75g and 100g), whereas *Paspalum distichum* L. had a relatively milder effect. Application of 75g and 100g of *Calotropis procera* completely inhibited the germination of wheat and growth and subsequently adversely affected other important growth attributes. However, the better growth of wheat in terms of germination, final emergence, and seedling length, fresh and dry weight of root and shoot, and chlorophyll content was observed in control (where no leaf litter was applied). In crux, the presence of invasive weeds in the field substantially hampers and inhibits wheat growth through Allelopathic interference and should be managed carefully in field crops.

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Novelty Statement

Incorporation of weeds and its stubbles are common practice in mechanical weeds management. Calotropis procera and Paspalum disticham are common found in soils of D.I.Khan. By studying their leaves litter contamination and incorporation into soil, their allelopathic and phytotoxic effect have determined at different concentration. It will contribute to knowledge about phytotoxicity of these two invasive weeds and their weeds management.

Author's Contribution

Muhammad Asad: Research scholar.
Iqtidar Hussain: Principal Investigator + Technical help + Overall management of the article
Faisal Nadeem: Co-principal Investigator + Data collection
Hafiz Muhammad Jhanzab: Co-principal Investigator +Data collection
Muhammad Saad and Muhammad Saqib: Data Analysis
Rashid Khan: Did SPSS analysis
Muhammad Safdar Baloch: Technical Input.

Shumaila Kiran and Salma Shaheen: Data collection + Data analysis.

Generative AI and AI-assisted technology statement
No Generative AI or AI assisted technology was used while writing manuscript or for analyzing result.

Conflict of interest
The authors have declared no conflict of interest.

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