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Phytotoxic Effects of Selected Weeds Associated with Wheat Crop

[a]Anum Saeed, [a]Madiha Rashid*, [b]Muhammad Aslam, [a]Muhammad Iftikhar, [a]Noreen Kareem, [a]Anam Ilyas, [a]Faiza Idrees, [a]Zahid Mehmood, [a]Jamil Aslam, [a]Nageen Hanif

a Department of Botany, Division of Science and Technology, University of Education Lahore, Pakistan.
b Department of Chemistry, Division of Science and Technology, University of Education Lahore, Pakistan.

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ABSTRACT

Weeds associated with various crops can significantly affect the growth of surrounding plants through chemical interaction. Understanding the phytochemical profile of these weeds and their potential allelopathic effects is crucial for developing effective weed management strategies. Therefore, the study was conducted to evaluate the phytotoxic effects of *Stellaria media*, *Amaranthus viridis*, *Euphorbia helioscopia*, and *Fumaria indica* on the growth and germination of wheat. Four different extracts (i.e., methanol, ethanol, acetone, and aqueous) of all plant species were analyzed for the allelopathic potential and screening of essential phytochemicals. Alkaloids, saponins, and terpenoids were found in all species. Phlobatannins were also present in all species except *Convolvulus arvensis.* Allelopathic evaluation of all these extracts was done against wheat by filter paper method. Two different concentrations (0.4% and 0.6%) for each of the extracts were used for this evaluation. The inhibitory effect on seed germination was increased with increasing concentration of the extract. Results have indicated the inhibitory effect of *Stellaria media, Amaranthus viridis*, *Euphorbia helioscopia,* and *Fumaria indica* on seed germination of wheat at all concentrations. While maximum growth retardation (98%) was displayed by ethanol extract of *Stellaria media.* Results concluded that *Stellaria media* had direct inhibitory effects on the growth of wheat. Therefore, its incorporation in an intercropping system should be avoided. Moreover, identification of major phytochemicals responsible for the allelopathic potential of these species needs further investigation before imposing them on agricultural practices.

Corresponding Author: Madiha Rashid
Email: madiha.rashid@ue.edu.pk

INTRODUCTION

Allelochemicals are secondary metabolites that have bioherbicide and growth-regulating properties (Anwar et al., 2021). Plants produce a variety of such kinds of secondary metabolites as a by-product of different metabolic reactions (Ofoedum *et al*., 2023). Terpenes, terpenoids, phenols, alkaloids, and flavonoids are the most important among them (Hilal *et al*, 2024). These metabolites can be found in any plant part, i.e., bark, flowers, roots, fruit, and seeds (Ahmed *et al*., 2024). Their presence and concentration may vary from species to species as well as part to part within a single species. These phytochemicals are directly associated with different activities of plants like antioxidant, antimicrobial, and other medicinal activities (Ashraf *et al*., 2023). Terpenoids are found to be involved in anti-inflammatory, anticancer, antimalarial, and antiviral activities (Cheraghabadi *et al*., 2023). Polyphenolic compounds have been reported for their anti-carcinogenic and anti-mutagenic effects. Flavonoids exhibited antibacterial activities (Wang *et al*., 2023).

The presence of phytochemicals in different concentrations is also the reason why plants have been considered a source of several medicines for thousands of years (Abdallah *et al*., 2023). Roots of *Convolvulus arvensis* are considered a laxative and are also used for flu, cough, and skin diseases (Ali *et al*., 2013). *Fumaria indica* is responsible for its antipyretic activity (Ali *et al*., 2020). It is reported that *Stellaria media* have polyphenols and flavonoids with antioxidant capacity for wound healing Miere *et al*. (2021). It has also been reported for its anti-inflammatory and anti-pyretic activities (Anyiam *et al*., 2023). *Euphorbia helioscopia* is used for the cure of cholera (Waheed *et al*. 2020). Extracts of *Amaranthus viridis* exhibited anti-diabetic and anti-cholesterolemic activities (Campos *et al*., 2024). Phytochemicals associated with different plants might have some effects on other plants showing their allelopathic potential. These allelochemicals might affect their surrounding plants either positively or negatively (Zhang *et al*., 2021). Allelochemicals are reported to be present in all plant parts including stem, leaves, roots, pollen grains, etc. (Ain *et al*., 2023).

One of the most significant staple crops that significantly contributes to the world's food security is wheat (*Triticum aestivum*) (Shiferaw *et al*., 2013; Francesconi *et al*., 2021). Twenty percent of global human nutritional calories are generated by it Wu *et al*., (2021). More than 100 distinct diseases that are brought on by different sources target wheat. Those diseases cause about 21.5% of wheat yield to be lost annually Savary *et al*., 2019. Global wheat production must be increased to meet the future demands imposed by population and prosperity growth (Li *et al*., 2024). Allelopathic effects of different weeds have been reported on wheat crop (Ullah *et al*., 2023; Vashishth *et al*., 2023; Khan *et al*., 2023).

In recent years, many plants have been screened for the presence of phytochemicals and their effects on other plants (Saxena *et al*., 2023; Nuryandani *et al*., 2024; Muscolo *et al*., 2024). Deepti *et al*. (2023) studied the allelopathic effects of Euphorbiaceae members on germination and seedling growth of wheat were reduced due to the presence of secondary metabolites. Moreover, allelopathic effects of various weed extracts on seed germination of 11 crop species were reported by Kadioglu *et al*. (2005). Despite a large number of reports, limited studies are available on phytochemical screening of weeds associated with wheat crop, along with their allelopathic potential. Therefore, the study was commenced to work on a preliminary screening of five common medicinal species (*Convolvulus arvensis, Amaranthus viridis, Stellaria media, Euphorbia helioscopia,* and *Fumaria indica*) and their allelopathic effects on wheat crop.

MATERIALS AND METHODS

The experiments were conducted to screen five medicinal species i.e., *Convolvulus arvensis* L., *Amaranthus viridis* L., *Stellaria media* L., *Euphorbia helioscopia* L., and *Fumaria indica* (Hausskn.) Pugsley on the basis of their phytochemicals and their allelopathic potential on seed germination and seedling growth of *Triticum aestivum* L. (wheat). Healthy seeds of wheat were collected from Punjab Seed Corporation, Pakistan. After collection, the seeds were surface-sterilized for ten minutes in a solution containing 1% sodium hypochlorite and then rinsed with distilled water. Whole plants of the above-mentioned species were collected, washed, air dried, and ground into fine powder and stored in dry condition until used. To prepare the aqueous extracts, 20 g of fine powder of each donor species was weighed and extracted in 100 ml of distilled water, by hot water extraction method (Shadab *et al*., 2024). Maceration technique (Andriani *et al*., 2023) was used to prepare the organic solvent extracts. 20 g fine powder of each species was soaked in 100 ml of methanol, ethanol, and acetone separately kept overnight, and then filtered to get the extracts.

Preliminary phytochemical screening

The aqueous and organic solvent extracts of all plant species were subjected to following chemical tests of different phyto-constituents using standard procedures (Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973).

Test for tannins

About 0.5 g of the crude extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration (Tease and Evans, 1989).

Test for alkaloids

Crude extract was mixed with 2 ml of Wagner's reagent. A reddish brown-colored precipitate indicates the presence of alkaloids (Ushie *et al*., 2022).

Test for phlobatannins

Crude extract of each plant sample was boiled with 2% aqueous HCl. The deposition of a red precipitate was taken as evidence of the presence of phlobatannins (Azuaga *et al*., 2022).

Test for flavonoids

5 ml of dilute ammonia solution was added to a portion of the crude extract followed by the addition of concentrated H_2SO_4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing (Fachriyaha *et al*., 2020).

Test for saponins

Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication of the presence of saponins (Góral *et al*., 2020).

Test for quinones

Dilute NaOH was added to 1 ml of crude extract. Blue, green, or red coloration indicates the presence of quinones (Goel *et al*., 2020).

Test for coumarin

10 % NaOH was added to the extract and chloroform was added for observation of yellow color, which shows the presence of Coumarin (Lončar *et al*., 2020).

Test for terpenoids

(Salkowski Test) 5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish-brown coloration of the interface was formed to show positive results for the presence of terpenoids (Łukowski *et al*., 2020).

Test for steroids

2 ml of acetic anhydride was added to 0.5 ml crude extract of plant sample with 2 ml of H_2S0_4. The color change from violet to blue or green in samples indicates the presence of steroids (Fardiyah *et al*., 2020).

Test for carbohydrates

(Fehling's Test) An equal volume of Fehling A and Fehling B reagents were mixed and 2ml of it was added to crude extract and gently boiled. A brick-red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars (McCleary *et al*., 2020).

Test for proteins (Millon's test)

Crude extract when mixed with 2ml of Millon's reagent, white precipitates appeared which turned red upon gentle heating that confirmed the presence of proteins (Yadav *et al*., 2011).

Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicates the presence of phenols and tannin (Tamilselvi *et al*., 2012).

Test for glycosides (Liebermann's test)

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A color change from violet to blue to green indicates the presence of a steroidal nucleus, i.e., the glycine portion of glycoside (Osman *et al*., 2014).

Evaluation of allelopathic potential

The aqueous and organic solvent extracts of all the species were evaluated for their allelopathic potential. Filtrate of aqueous and organic solvent extracts were further diluted into 0.4% and 0.6% concentrations. Wheat seeds were used to screen the allelopathic potential of donor species by filter paper method, due to their effectiveness and ease of handling. Filter paper is the best medium for the germination of seeds as it provides porosity for airflow and absorbance of plant extract (Greuning *et al*., 1998). Different extracts of 0.4% and 0.6% concentrations were applied to test plants by soaking a double layer of filter papers. Ten seeds of the test plant were placed between two folds of filter paper in each petri dish. Extracts of each plant species were applied to seeds. Distilled water was used for the control set. The experiment with each treatment was replicated thrice. After seven days, data was recorded for germination and growth in the form of root and shoot length.

Data analysis

Data was analyzed by SPSS 13.0 (Statistical Package for the Social Sciences) and Microsoft Excel. Single-factor ANOVA was performed to investigate the significance of activity. Level of significance was 0.05. Data for the presence or absence of phytochemicals, percentage germination, and growths of radicle and hypocotyl were represented by bar and line graphs (Bhuker *et al.*, 2023).

RESULTS

Experiments were conducted to screen the phytoconstituents and allelopathic potential of *Convolvulus arvensis* L., *Amaranthus viridis* L., *Stellaria media* L., *Euphorbia helioscopia* L. and *Fumaria indica* (Hausskn.) Pugsley. Different extracts i.e. methanol, ethanol, acetone, and aqueous were used for qualitative analysis of phytochemicals as well as for allelopathic potential against the germination and growth of wheat as a test plant.

Phytochemical screening

For qualitative analysis, several phytochemical tests were performed to check the presence or absence of different phytoconstituents. Results indicated the presence of tannins, alkaloids, flavonoids, saponins, coumarins, quinones, phlobatannins, carbohydrates, proteins, phenols, terpenoids, steroids, and glycosides in different plants species (Table 1).

Table 1. Phytochemical Analysis of Different Plant Species.

Tests	Different Plant Species				
	Convolvulus arvensis	*Stellaria media*	*Fumaria indica*	*Euphorbia helioscopia*	*Amaranthus viridis*
Tannins	+	-	+	+	+
Alkaloids	+	+	+	+	+
Flavonoids	+	+	-	+	+
Saponins	+	+	+	+	+
Quinones	-	+	+	-	-
Coumarins	+	-	+	+	-
Terpenoids	+	+	+	+	+
Steroids	+	-	-	+	+
Carbohydrates	+	+	+	+	+
Proteins	+	+	+	+	+
Phenols	+	+	+	+	+
Glycosides	+	-	+	+	+
Phlobatannins	-	+	+	-	+

Evaluation of allelopathic potential

Various effects were detected under the influence of different concentrations of plant extracts. Donor plants displayed both stimulatory and inhibitory effects on seed germination of the test plant. Ethanol extract of all plants showed inhibition of wheat at its higher concentrations. In case of *Amaranthus viridis*, maximum germination inhibition (17%) was observed at 0.6% concentration of aqueous extract, followed by ethanol extract, 14% inhibition. Acetone and aqueous extract of *Stellaria media* had negligible effects on seed germination of the test plant. All extracts of *Convolvulus arvensis* showed stimulation in seed germination except ethanol extract. *Fumaria indica* inhibited seed germination of wheat at all of its concentrations. By increasing the extract concentration, inhibition was also increased. Ethanol and acetone extracts of *Euphorbia helioscopia* showed 17% stimulation in all of its concentrations. Methanol extract showed 27% and 20% stimulation at 0.4% and 0.6% concentrations respectively. Aqueous extract enhanced 20% seed germination at its 0.4% concentration.

Acetone extract of *Euphorbia helioscopia* showed maximum stimulation (19%) in seedling growth of wheat plant while maximum growth retardation (98%) was displayed by ethanol extract of *Stellaria media.* Results have indicated more retardation in radicle parts as compared to hypocotyl. Minimum retardation (7%) was displayed by acetone extract of *Stellaria media.* ANOVA showed significant results (0.01) with methanol extract. Stimulation in seedling growth was exhibited by *Amaranthus viridis* and *Euphorbia helioscopia.* Moreover, 83% growth suppression was recorded in hypocotyl of the test plant by methanol extract (0.6%) while 90% inhibition was seen in radicle growth at the same concentration of *Amaranthus viridis*. ANOVA showed significant differences i.e., 0.0028 (Figure 2; Table 2). All extracts of *Convolvulus arvensis* inhibited the growth of wheat seedlings except acetone extract. Maximum inhibition (6%) was shown by aqueous extract (0.4%) while maximum stimulation (10%) was shown by acetone extract at the same concentration. Ethanol extract at both concentrations i.e. 0.4% and 0.6%, showed 54% growth suppression of radicle of wheat. Up to 89% growth retardation was observed by aqueous extract of *C. arvensis.* Significant results were observed with methanol and ethanol extracts (Figure 1 and 2; Table 2).

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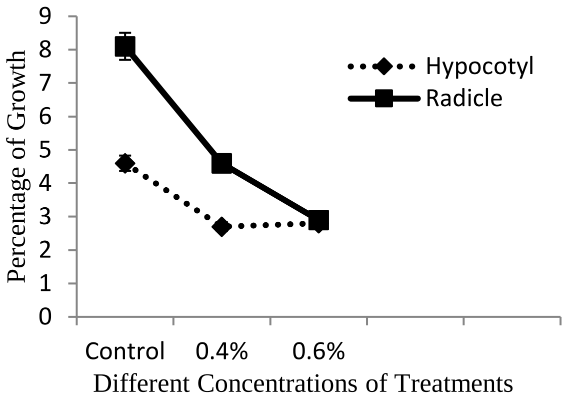
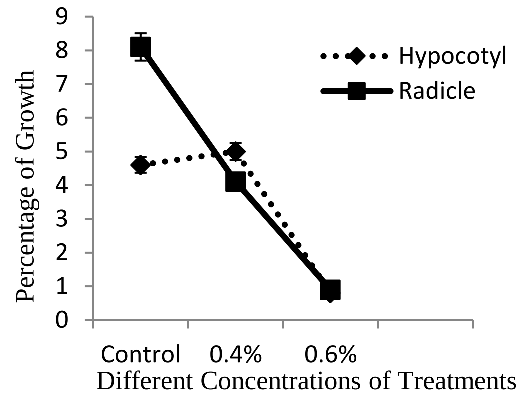
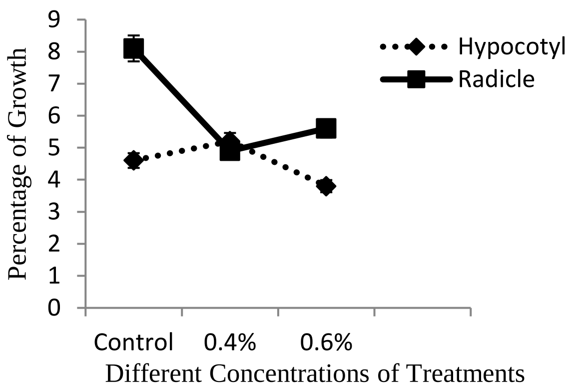


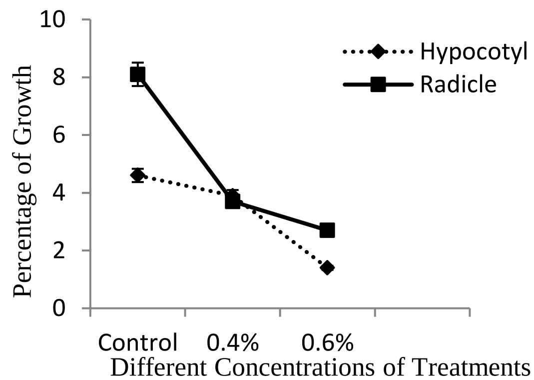
(a) Aqueous Extract of *Amaranthus viridis*



(b) Methanol Extract of *Amaranthus viridis*

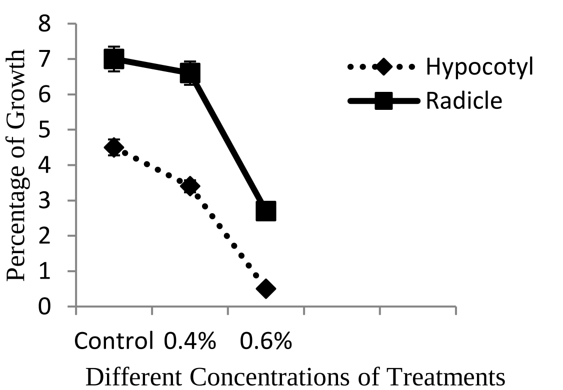


(c) Acetone Extract of *Amaranthus viridis*

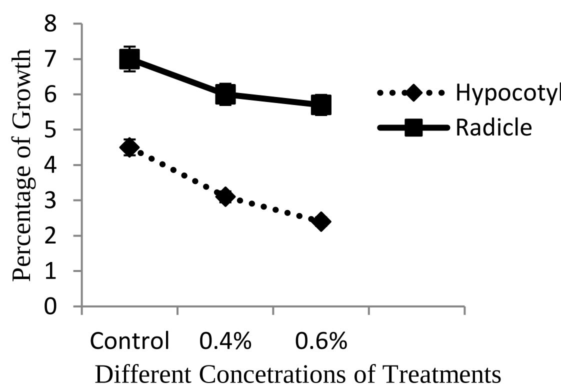


(d) Ethanol Extract of *Amaranthus viridis*

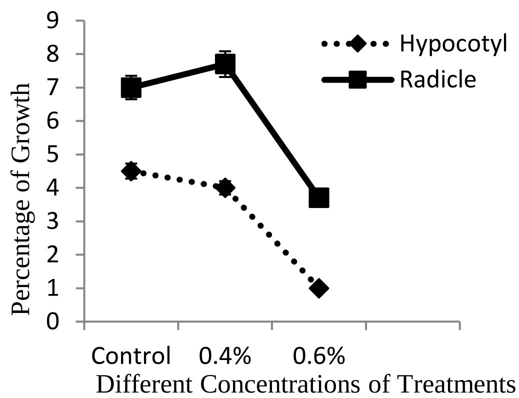
Figure 1. Effect of *Amaranthus viridis* extracts on growth of Wheat.



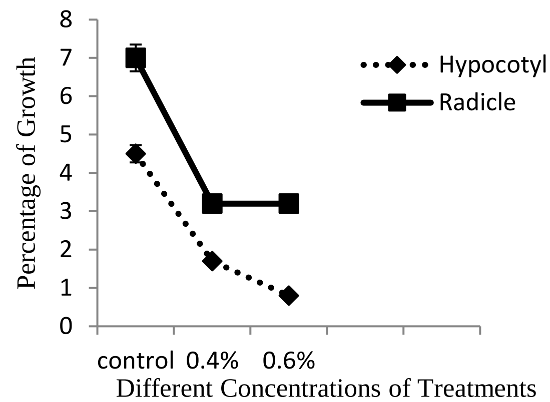
(a) Aqueous Extract of *Convolvulus arvensis*



(b) Methanol Extract of *Convolvulus arvensis*



(c) Acetone Extract of *Convolvulus arvensis*



(d) Ethanol Extract of *Convolvulus arvensis*

Figure 2. Effect of *Convolvulus arvensis* extracts on growth of Wheat.

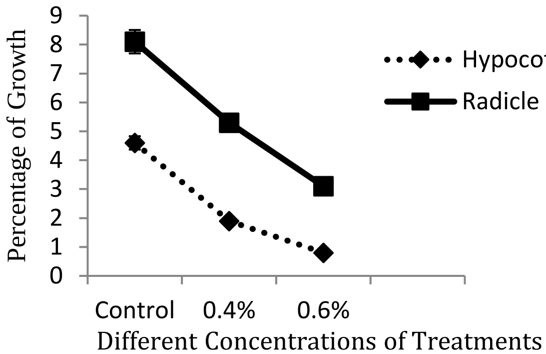
All extracts of *Fumaria indica* showed diverse inhibitory effects on the growth of wheat. Maximum growth inhibition (94%) was exhibited by ethanol extract of the donor plant. By increasing the concentration of extract, growth inhibition was also increased. About 85% of hypocotyl growth was suppressed by methanol extract of the same plant. Results have shown significant effects of all the extracts on the growth of wheat. In case of *Euphorbia helioscopia*, maximum growth suppression (80%) was shown by its ethanol extract. The effects of all the extracts were found to be highly significant. Aqueous extract of *Stellaria media* was found to be inhibitory, up to 89% while ethanol extract suppressed the growth to 98%. The results were also found to be highly significant (Figure 3, 4 and 5; Table 2).

Table 2. Allelopathic effect of different plant species on the growth of Wheat.

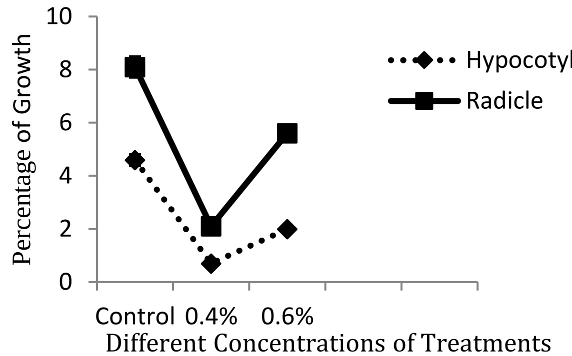
Plant Species	Extracts	Control		Length of Hypocotyl and Radicle in Different Concentrations				P Value	
				0.4 %		0.6 %			
		H	R	H	R	H	R	H	R
Amaranthus viridis	Methanol	4.6	8.1	5	0.8	0.9	0.9	0.00*	0.00*
	Ethanol	4.6	8.1	3.9	3.7	1.4	2.7	0.00*	0.00*
	Acetone	4.6	8.1	5.2	4.9	3.8	5.6	0.0028*	0.00*
	Aqueous	4.6	8.1	2.7	4.6	2.8	2.9	0.00025*	0.00*
Convolvulus arvensis	Methanol	4.5	7	3.1	6	2.4	5.7	0.0002*	0.00*
	Ethanol	4.5	7	1.7	3.2	0.8	3.2	0.008*	0.00*
	Acetone	4.5	7	4	7.7	1	3.7	0.00*	0.00003*
	Aqueous	4.5	7	3.4	6	0.5	2.7	0.00*	0.00*
Fumaria indica	Methanol	4.6	8.1	0.7	2.1	2	5.6	0.00*	0.00*
	Ethanol	4.6	8.1	0.5	2.8	0.3	0.6	0.00*	0.00*
	Acetone	4.6	8.1	0.9	2	1.3	3.3	0.00*	0.00*
	Aqueous	4.6	8.1	1.9	5.3	0.8	3.1	0.00*	0.00*
Euphorbia helioscopia	Methanol	3.4	4.3	3.7	1.6	0.5	1.7	0.0002*	0.00002*
	Ethanol	3.4	4.3	0.7	3.5	1.3	2	0.000003*	0.003*
	Acetone	3.4	4.3	3.4	5.1	0.9	1.9	0.004*	0.0002*
	Aqueous	3.4	4.3	2.7	4.8	2.4	4.3	0.00006*	0.03**
Stellaria media	Methanol	4.7	8.2	2.5	5.5	3.8	6.1	0.01**	0.00004*

	Ethanol	4.7	8.2	3.3	5.7	0.1	0.7	0.0005*	0.0003*
	Acetone	4.7	8.2	3.8	7.6	1.3	3.6	0.0001*	0.00*
	Aqueous	4.7	8.2	3.4	6.4	0.5	2.8	0.01**	0.0003*

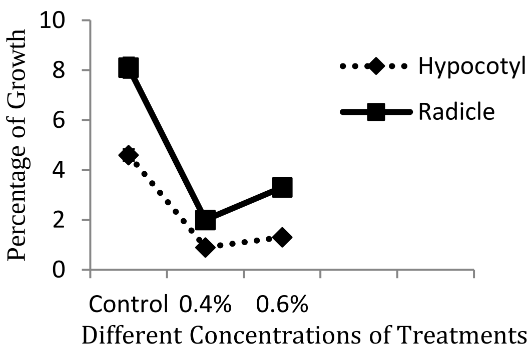
Key: H = Hypocotyl, R = Radicle, * = Significant Effect, ** = Non-significant Effect, Alpha Value: 0.05



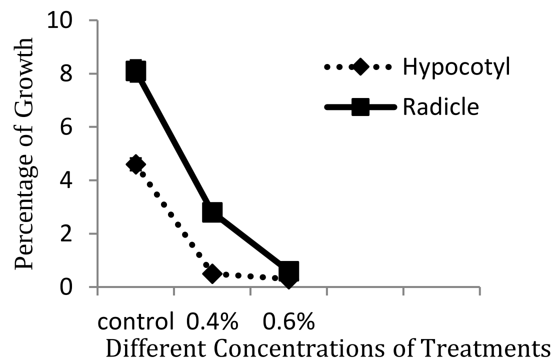
(a) Aqueous Extract of *Fumaria indica*



(b) Methanol Extract of *Fumaria indica*

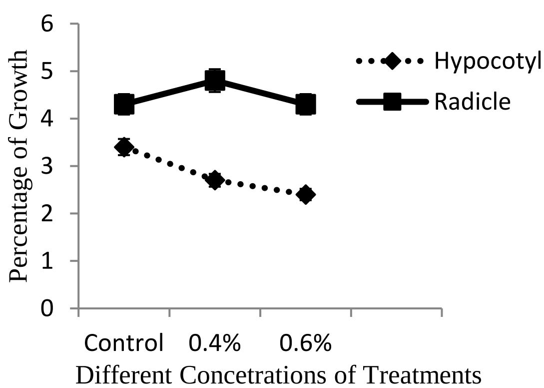


(c) Acetone Extract of *Fumaria indica*

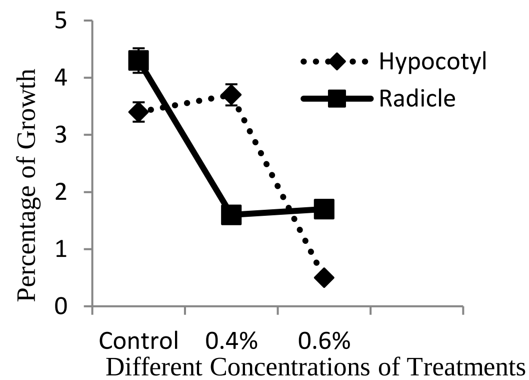


(d) Ethanol Extract of *Fumaria indica*

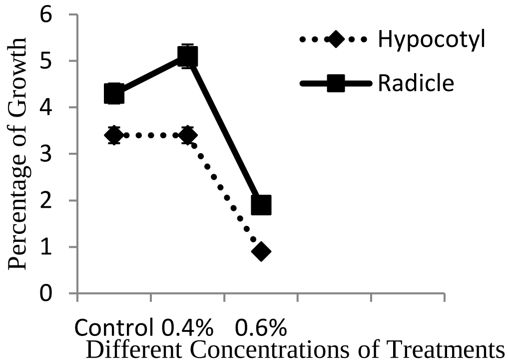
Figure 3. Effect of *Fumaria indica* extracts on Growth of Wheat.



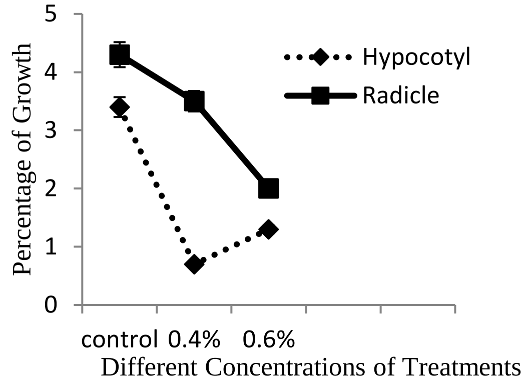
(a) Aqueous Extract of *Euphorbia helioscopia*



(b) Methanol Extract of *Euphorbia helioscopia*

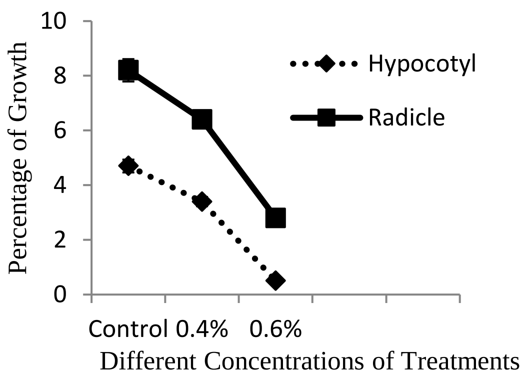


(c) Acetone Extract of *Euphorbia helioscopia*

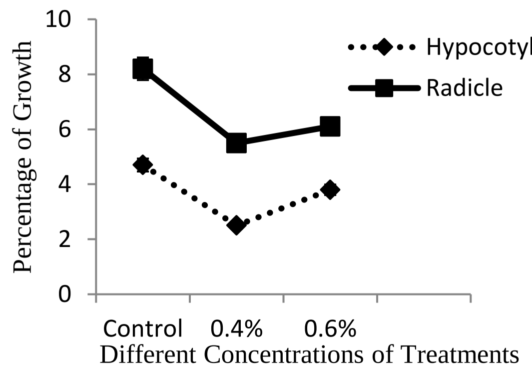


(d) Ethanol Extract of *Euphorbia helioscopia*

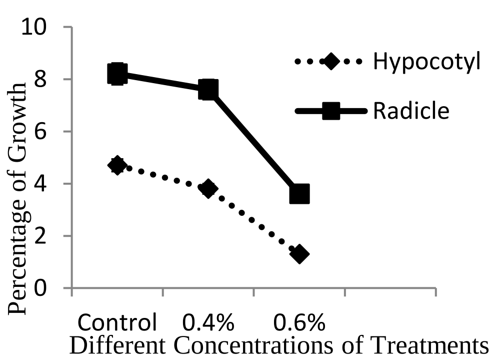
Figure 4. Effect of *Euphorbia helioscopia* extracts on growth of Wheat.



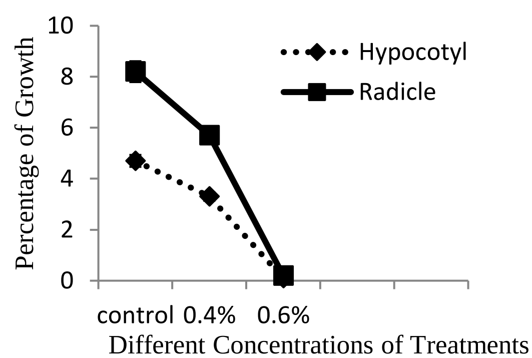
(a) Aqueous Extract of *Stellaria media*



(b) Methanol Extract of *Stellaria media*



(c) Acetone Extract of *Stellaria media*



(d) Ethanol Extract of *Stellaria media*

Figure 5. Effect of *Stellaria media* extracts on growth of Wheat.

DISCUSSION

Chemicals released from plants that can impose allelopathic influences on their surrounding plants are termed allelochemicals. Most allelochemicals are classified as secondary metabolites (Bachheti *et al*., 2023; Hickman *et al*., 2021). The secondary metabolites from plants are important sources of potentially useful growth stimulators and inhibitors (Nephali *et al*., 2020). These secondary metabolites (also called allelochemicals) might be present in all plant parts and tissues with different concentrations (Scognamiglio *et al*., 2020; Hoang *et al*., 2021). In the present results, alkaloids, phlobatannins, flavonoids, saponins, quinones, and terpenoids were found in all extracts of *Stellaria* *media.* These findings were supported by the observations of Bukola et al. (2011) whose study also reported the presence of phlobatannins and saponins in *S. media*.

Many researchers have reported their work in the field of allelopathy (Li et al., 2021; Gurmani et al., 2021; Bashar, et al., 2023). When susceptible plants are exposed to allelochemicals; germination, growth, and development might be affected. The most frequently reported gross morphological effects on plants were inhibition or stimulation of seed germination, coleoptiles elongation, and development of shoot and root (Pucciariello et al., 2020). In the present work, *Euphorbia helioscopia* had a stimulatory effect on seed germination at all of its concentrations. The results of the present study corroborated the findings of Tanveer *et al*. (2010), who reported that *Euphorbia helioscopia* suppressed germination and seedling growth of lentils and chickpea to a much greater extent than that of wheat. Khalid *et al*. (2020) also observed the reduction of root length and shoot length due to the effects of *E. helioscopia* on the germination of wheat.

In the present study, *Convolvulus arvensis* stimulated the seedling growth of wheat at all concentrations. Similarly, Hegab and Ghareib (2010) also reported growth stimulation in wheat by methanolic extract of *Convolvulus arvensis*. Present findings have indicated maximum growth retardation in wheat seedlings by *Stellaria media.* These findings were in agreement with the results of Inderjit et al. (2014). They reported the potential of water-soluble phenolics of chickweed on wheat growth. Therefore, the growth retardation by *Stellaria media*, found in present results, might be due to the presence of phenols in it.

Present results have indicated more retardation in radicle parts as compared to hypocotyl. These results were consistent with the data of Chon et al., (2011). Akter et al. (2023) reported stronger inhibitory effects of 15 common weed species on roots as compared to shoots. The reduction in root length has also indicated the direct effects on cell elongation, as allelopathic agents were also found to be inhibitory towards gibberellin production (Shohat *et al*., 2021). High concentrations of extracts usually have the highest toxicity on seed germination (Suksungworn *et al*., 2016). Similarly, in the present results, ethanol extract of *Fumaria indica*, at its higher concentration, was more inhibitory towards the growth of the test plant.

CONCLUSION

The study has disclosed the presence of tannins, alkaloids, flavonoids, saponins, coumarins, quinones, phlobatannins, carbohydrates, proteins, phenols, terpenoids, steroids, and glycosides in different extracts of donor plants. Alkaloids, saponins, and terpenoids were found in all plants. Phlobatannins showed their presence in all plants except *Convolvulus arvensis.* Results of allelopathic activities have shown that ethanol extracts of all donor plants exhibited more inhibitory effects against the test plant (wheat) at its different concentrations. *Stellaria media* has a stimulatory effect at lower concentrations and while inhibitory effect at higher concentrations. On the other hand, *Fumaria indica, Euphorbia helioscopia,* and *Amaranthus viridis* had inhibitory effects on seed germination at all of their concentrations. Based on these results, it was concluded that *Fumaria indica, Euphorbia helioscopia,* and *Amaranthus viridis* possessed phytotoxic effects, therefore, it is necessary to keep these weeds under check at the emergence stage so that growth suppression of crops could be avoided. Moreover, the identification of major chemicals responsible for allelopathic potential requires further phytochemical investigation before imposing them on agricultural practices.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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