Research Article

Allelopathic Effects of *Verbesina encelioides* Extracts on the Germination and Growth Parameters of Monocotyledonous Seeds

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Abstract | A lab study was conducted to investigate the ability of *Verbesina encelioides* at different concentrations on wheat and maize seeds at the Botany Laboratory Government Girls Degree College KDA Karak. Eight different concentrations of *V. encelioides* leaves were applied on maize and wheat seeds to check the inhibitory and stimulatory effect, respectively. Whole plant of *V. encelioides* was collected, dried, powdered, and aqueous extracts were prepared at room temperature. Randomized Complete Design (RCD) with four treatments and one control were used in the experiment. Data was recorded for seed germination rate (%), shoot length (cm), radical length (cm), fresh biomass weight (g), dry weight (g), and moisture content (%) of wheat and maize seedling. Result revealed that *V. encelioides* extracts significantly affected all the growth variables of wheat and maize seeds. Results revealed that *V. encelioides* has prominent allelopathic potential against the tested species.

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Introduction

Allelopathy is the study of interaction of plants with other plants or with other organisms (microorganisms) through the release of chemicals (secondary metabolic products produce in plants), known as allelochemicals (Weir *et al.*, 2004). Numerous researchers have been investigated the allelopathic potentials of different plants, however no literature review is available on the inhibitory effect of *V. encelioides* on vegetables or cereals. Allelopathy reduces the environmental impact of agriculture while enabling sustainable weed management (Vincenzo *et al.*, 2008). There are certain plants which has considerable allelopathic ability. *V. encelioides*, in the present study, is investigated for its allelopathic and hence weed controlling potential.

Verbesina encelioides

V. encelioides (Cav.) Benth and Hook, a member of the Asteraceae family, is commonly called as golden crown beard, propagates quickly through winged seeds, produced in a very huge amount (Sayari *et al.*, 2016). The plant has been reported as a cause of galegine poisoning in livestock when drinking water is being contaminated by dried *V. encelioides* plant (Lopez *et*

al., 1996). Flower and leaf extracts of *V. encelioides* have been reported to be more toxic than its root leachates and stem extracts (Goel, 1987), and studies have proved that various secondary metabolites are the cause of its toxicity. Moreover, pharmaceutical industry has found use of this plant's various vegetative parts and flower as anti-tumor, anti-viral and anti-microbial characteristics (Jain *et al.*, 2007).

Triticum aestivum

Triticum aestivum (wheat) of family Poaceae is the source of providing staple food to more than 50 % world population and due to its divers use in food, it is often referred to as the "king of cereal crops" in Pakistan (Rahman *et al.*, 2014). Although it originated in the Ethiopian Highlands, currently it is grown all over the world. With 3.72% of the world's wheat-producing area, Pakistan ranks eighth in terms of global wheat output. Apart from other reasons responsible for losses in wheat production, weeds are a major threatening factor due to their competitive and allelopathic character and roughly causes 17 to 25% annual losses in wheat yield according to agricultural specialists (Shah *et al.*, 2006).

Zea mays

Zea mays or commonly the corn or maize, of *Poaceae*, is among the significant annual grain crop, ranked as third-largest crop used as staple food worldwide following rice and wheat (Sandhu *et al.*, 2007). It is considered as one of the most significant crops cultivated in both irrigated and rainfed environments. In Pakistan at national level, the area under maize cultivation is 1653000 hectares having 16.6 % increase recorded during 2021-2022 (MNFSR, 2022). As in the case of other cereal crops, Maize too faces losses in yields due to weed competitions besides other agricultural/environmental factors.

Materials and Methods

Collection of plant material

In April 2021, healthy *V. encelioides* plants were harvested from the KDA region from Karak.

Seed collection

Seeds of *Triticum aestivum* and *Zea mays* were collected from certified seed center in Karak.

Extract preparation of V. encelioides

The whole plant *V. encelioides* was washed thoroughly and shade dried for two weeks and converted to fine powder by electric grinder. Powder was soaked in water and methanol to prepare aqueous extracts and methanol extracts. Extracts were filtered and kept open for evaporation of extra ethanol and then refrigerated at 4°C for further experiments.

Parameters to be studied

During the experiment, the ensuing variables were examined.

1. Germination Percentage: After third day of sowing, germinated seeds were counted, and germination percentage was noted.
2. Radicle length (cm): Germinated seeds, root length was measured.
3. Plumule length (cm): Germinated seeds, plumule length was measured.
4. Seminal roots, count: seminal roots were counted.
5. Weight(gm) of fresh biomass: 10 seeds were randomly selected, and weight was measured in grams.
6. Dry weight(gm): the 10 seeds chosen for fresh biomass determination were dried in ovan randomly and weight was measured in grams.
7. Moisture content (%): Moisture content was calculated with the help of formula.

Aqueous extracts treatment

5 and 10 gm of the whole plant was socked in 100ml distilled water at 25°C for 24 hrs and filtered to get the aqueous extracts. These extracts were tested against wheat and maize, on two folds of filter paper in Petri dishes. The filter papers were moistened with the respective extracts and with distilled water in control e. Data on germination, plumule and radical growth was recorded after 72 hours. Ten germinated seeds were randomly selected for fresh biomass and dry weight determination. Seedlings were dried at 65°C for 72 hours.

Hot water extracts treatment

5 and 10 gm of the whole plant were separately boiled in100ml of distilled water for 10 minutes and filtered. The filtrate was cooled at room temperature and applied against the same test species as before.

Litter bed treatment

5gm powdered material of the whole plant was placed in Petri dishes, two layers of filter papers were placed on it and moistened with 5ml distilled water.

In control, distilled water moistened filter paper was used. The experiment was repeated as in aqueous and hot water bioassay.

Methanol extract treatment

Methanol extract was prepared by socking five gm and ten gm powdered plant material in 100ml methanol, separately. The extract was regularly shaked vigorously for 15 days, and then the semi solid residue was obtained by evaporating extra solvent from filtrate used for seeds irrigation. The experiment was repeated as in aqueous and hot water bioassay.

Rhizosphere experiment

To check the effect of rhizosphere soil on seed germination and growth parameters of the tested species, the root zone area of *Verbesina* was dug out and soil was collected from it. For control soil from a nearby locality, with no-vegetation, was collected. After necessary measures, both rhizosphere and control soils were put in clean sterilized pert dishes. The experiment was repeated as in aqueous and hot water bioassay and the data was taken after 7 days.

Statistical analysis

Data was analyzed using the ANOVA one way technique and student t-test where applicable by using Statistics 8.1 package. Wherever the F value was found significant Least significance difference (LSD) test was applied, at 5% level of probability to differentiate the treatments.

Results and Discussion

Crops importance, weeds harmful effects, need of methods of weeds eradication, Allelopathy is used as a tool for exploration of ecofriendly weedicides. May plants have been explored till now. As less literature was available on the Allelopathy of *V. encelioides*. So, in the present study this plant was studied for its allelopathic potential. The tested species were inhibited significantly in various vegetative parameters.

Wheat and maize seeds germination rates and other growth metrics were affected differently by different concentration of *Verbesina*s cold and hot aqueous extracts, methanol extract and in litter bed bioassay. Extracts effect was found concentration dependent as well as plant specific as is shown by results.

The recorded germination rate was 4%, 7.6%, 3.6%, and 4% for wheat seeds and 7.2%, 6.2%, 6.6% and 3.2% for maize seeds, by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. However, germination rate was 3.6%, 6.2%, 1.8% and 3.2% for wheat seeds and 4.6%, 4%, 5%, 2.6% for maize seeds, for 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. Tables 1 and 3; Figures 1 and 3 shows clearly control germination which was 10% each for wheat and maize. The finding is in line with the study finding of Muzzo *et al.* (2018) that elaborate that the leaf extract of *C. odorata* inhibited with two characteristics that are seed germination and seedling growth of some varieties of cowpea and pasture species.

Table 1: *Allelopathic effect of different concentration of V. encelioides on morphological attributes of wheat.*

Treatment/ Parameters	Seed germination %	Plumule length	Radical length	Seminal roots	Fresh weight	Dry weight	Moisture contents
Control	10	3.31	4.35	1.86	0.97	0.75	21.1
Cold water 5gm/24hrs	4	0.47	0.43	0.3	0.64	0.04	92.8
Cold water 10gm/24hrs	3.6	0.26	0.26	0.16	0.66	0.05	91.4
Hot water 5gm/24hrs	7.6	1.55	1.49	1.14	0.82	0.03	95.4
Hot water 10gm/24hrs	6.2	1	0.79	0.12	0.82	0.03	95.2
Methanol 5gm/24hrs	3.6	0.2	0.08	0	0.62	0.03	94.6
Methanol 10gm/24hrs	1.8	0.07	0.02	0	0.28	0.01	94.2
Litter Bed 5gm/24hrs	4	0.32	0.2	0.24	0.68	0.03	94.6
Litter Bed 10gm/24hre	3.2	0.28	0.27	0.08	0.7	0.03	94.4

Table 2: *Allelopathic effect of Rhizosphere soil of V. encelioides on morphological attributes of wheat.*

Treatment/ Parameters	Seed Germination %	Shoot length (cm)	Root length (cm)	Seedling height (cm)	No of leaves	Fresh weight	Dry weight	Moisture contents
Control	10	3.19	3.81	5.37	1.96	0.28	0.16	41
Rhizosphere soil	7.2	0.57	2.13	5.99	0.74	0.41	0.16	46.4

Table 3: *Allelopathic effect of different concentration of V. encelioides on morphological attributes of maize.*

Treatment/ Parameters	Seed germination %	Plumule length	Radical length	Seminal roots	Fresh weight	Dry weight	Moisture contents
Control	10	2.64	4.72	3.66	4.68	2.09	57.3
Cold water 5gm/24hrs	7.2	1.37	1.32	1.7	1.55	1	30.6
Cold water 10gm/24hrs	4.6	0.87	0.79	1.28	1.9	1.07	40.6
Hot water 5gm/24hrs	6.2	0.37	0.27	0	1.01	0.59	42
Hot water 10gm/24hrs	4	0.26	0.3	0	1.19	0.6	50.2
Methanol 5gm/24hrs	6.6	0.17	0.79	0.48	2.42	1.19	49.8
Methanol 10gm/24hrs	5	0.4	0.26	0.1	0.97	0.3	69.8
Litter Bed 5gm/24hrs	8.4	0.26	0.18	0.26	1.14	0.24	85.6
Litter Bed 10gm/24hre	2.8	0.24	0.13	0	1.4	0.26	84.8

Table 4: *Allelopathic effect of Rhizosphere soil of V. encelioides on morphological attributes of maize.*

Treatment/ Parameters	Seed germination %	Shoot length (cm)	Root length (cm)	Seedling height (cm)	No of leaves	Fresh weight	Dry weight	Moisture contents
Control	10	9.12	6.09	7.88	3.88	1.75	0.42	75.8
Rhizosphere soil	7.2	4.74	6.67	12.09	1.64	1.16	0.4	65.56

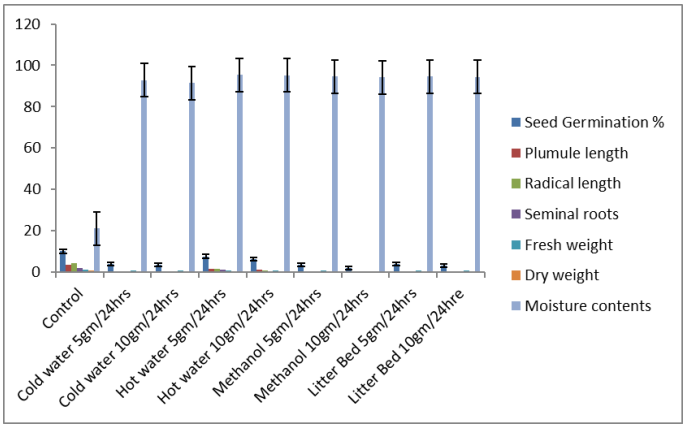

Figure 1: *Allelopathic effect of different concentration of Verbesina encelioides extract on morphological attributes of wheat.*

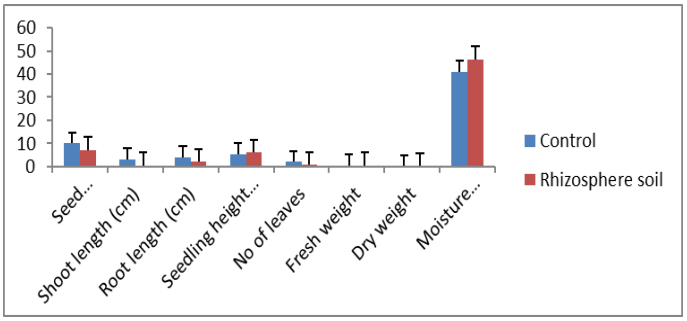

Figure 2: *Allelopathic effect of Rhizosphere soil of Verbesina encelioides extract on morphological attributes of wheat.*

The plumule length was 0.474cm, 1.55cm, 0.208cm, 0.324cm of wheat and 1.374cm, 0.37cm, 0.718 cm and 0.266cm of maize by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. For concentrated extract, the Plumule length was 0.266cm, 1.008cm, 0.7cm and 0.288cm for wheat and for maize it was 0.874cm, 0.26cm, 2.64cm and 0.24cm for 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. while in control the length of Plumule for wheat and maize was recorded 3.31 cm and 2.64 cm, respectively. These findings are in close resemblance with the study of Nadeem *et al.* (2020d) who studied the Phytotoxic effects of *Sonchus oleraceus* on emergence and seedling growth of *Echinocloa cruss-galli.* The results also coincide with the result of Julio *et al.* (2019) where it was revealed that inhibitory effect increases with extract concentration. For instance, the leaf extracts of *C. odorata* and *Lantana camara* suppressed the growth and germination of *Vigna radiata* seedlings. Hence, the inhibitory effect was directly proportional to extract concentration.

Similarly, Nadeem *et al.* (2021b) whose study was to investigate the Allelopathic potential of aqueous extracts of sow thistle weed on emergence and seedling growth of red rice, are in line with the current findings.

Radicle length was 0.432 cm, 1.49 cm, 0.79 cm, 0.202 cm of wheat and 1.328cm, 0.32cm, 0.88cm and 0.188 cm of maize by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay,

respectively. Radicle length was 0.374cm, 0.798cm, 0.026cm and 0.27cm for wheat and for maize it was 0.798cm, 0.302cm, 2.66cm and 0.138cm for 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. while in control the radicle length was 3.31cm for wheat and 4.74cm for maize.

The immobilization of significant amounts of nutrients by microorganisms involved in decomposition, by allelochemicals, or both may be the cause of the detrimental effects of plant leftovers on seed germination and plant growth, for instance.

It was observed during the study that number of seminal roots was different for both wheat and maize under the effect of various concentration. The same was noted for wheat 0.3, 1.14, 0.3 and 0 and 1.7, 0, 0.26 and 0.48 by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. Grand mean of total number of seminal roots was 0.16, 0.12, 0, and 0.8 for wheat and 1.28 ,0, 0.1, and 0 for maize by applying 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. While in control the grand mean of no seminal roots was 1.86 for wheat seeds and 3.6 for maize seeds.

Fresh biomass weight was 1.61gm, 1.44.gm, 4 gm and 2.85 gm for wheat and 5gm, 5 gm, 4gm and 4.77 gm for maize by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. Fresh biomass weight was 1.21gm, 1.11gm, 1.15 gm and 2.27 gm for wheat and 4gm, 4.15 gm, 3.8 gm and 3.11 gm for maize by applying 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. while in control the fresh weight for wheat and maize was 1.15gm and 4.9gm.

Dry weight (gm) was 0.92 gm, 0.98 gm, 0.75 gm and 1.45 gm for wheat and 2.67 gm, 3.15 gm, 2.19 gm and 2.14 gm for maize by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. For 10gm extract treated seeds was 0.88gm, 0.85 gm, 0.63 gm and 1.26 gm for wheat and 2.55 gm, 2.65 gm, 2.13 gm and 1.97 gm for maize by applying 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. while in control the fresh weight for wheat and maize was 0.75 gm and 2.09gm. Irfan *et al.* (2022) reported that the wild radish extract put devastating effects on the emergence and seedling development of turnip.

Moisture contents were 42.8 %, 48.2%, 51.9 %, and 49.1% for wheat seeds and47.6%, 37%, 45.2% and 55.1% for maize seeds, by applying 5gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. However, moisture contents were 27.2%, 36.2%, 45.2% and 44.4 % for wheat seeds and 36.2 %,36.1%,43.9%,36.6 % for maize seeds, for 10 gm cold water, hot water, methanol extract and in litter bed bioassay, respectively. while in control moisture contents was 34.7 % for wheat and 57.3% for maize, shown in Tables 1 and 3; Figures 1 and 3). The present findings are in line with the study by Javed *et al.* (2020), who investigated the herbicidal activity of *Ageratum conyzoides* against parthenium.

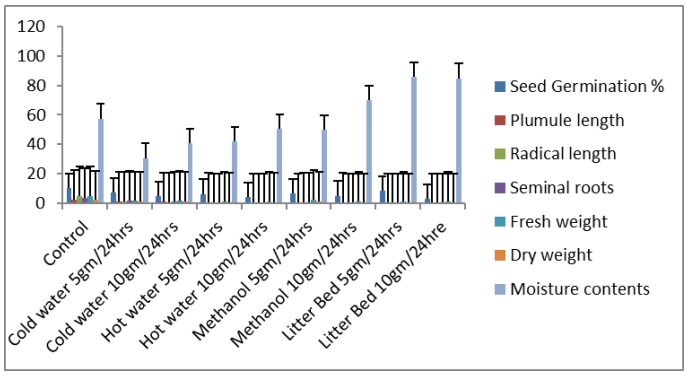

Figure 3: *Allelopathic effect of different concentration of Verbesina encelioides extract on morphological attributes of maize.*

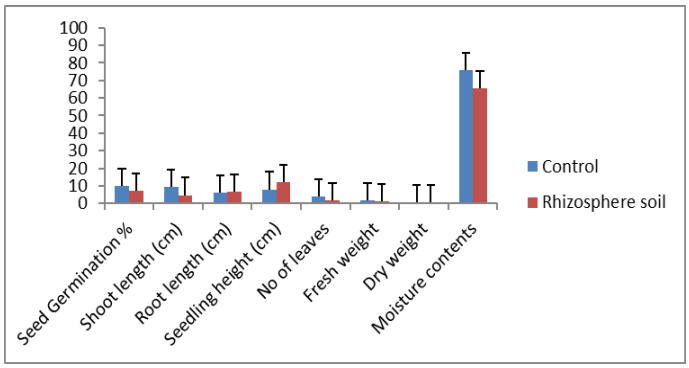

Figure 4: *Allelopathic effect of Rhizosphere soil of Verbesina encelioides extract on morphological attributes of maize.*

Rhizosphere soil effect on wheat and maize

Soil bed bioassay (rhizosphere) results clearly indicated the grand mean of germination for wheat in *V. encelioides* rhizosphere and similarly for maize seeds was 7.2% each. Tables 3 and 4 highlighted the controlling factor for germination of wheat seeds and maize which was also recorded 10% each. However,

the variation was found based on mean of the shoot length that was 0.57 cm for *Verbesina* rhizosphere grown wheat and 4.74 cm for maize seeds. Same variation recorded in control (plain soil from field) where a very clear difference was noted in shoot length that was 3.19 for wheat seeds and 9.12 cm for maize seeds. A clear variance based on root length was observed in rhizosphere soil for wheat seeds and maize seeds which 2.13 and 6.67 cm, respectively. In control, slightly higher difference was observed in grand mean of root length for wheat which was 3.81 while slightly low difference was observed for maize seeds i.e., 6.09 cm. As far as seedling height is concerned, the grand mean was recorded 5.99 cm for wheat seeds while for maize seed it was recorded 12.09 cm. In control, the seedling height was noted slightly low i.e., 5.37 cm for wheat seeds 7.88 cm for maize. Number of leaves for wheat was 0.74 in rhizosphere soil grown seeds while it was noted 1.64 cm for maize seeds. In control, the number of leaves was 1.99 cm for wheat seeds while it was recorded 3.88 for maize seeds. Rhizosphere soil grown seeds for wheat seeds and maize seeds variation was noted based on fresh weight which was 0.41 gm and 1.16 gm, respectively. Whereas, controlling the fresh weight for both wheat seeds and maize seeds were found 0.28 gm and 1.75 gm, respectively. Same parameters for wheat and maize seeds were analyzed based on dry weight, which was 0.16 gm and 0.4 gm, respectively, however, surprisingly result obtained in controlling the dry weight which as it was mentioned above i.e. 0.16 gm for wheat and 0.4 gm for maize seeds. Wheat seeds and maize seeds were analyzed based on moisture content, calculated from seeds grown in rhizosphere soil which was recorded as 46.4% for wheat seeds and 65.56% for maize seeds. In control, the moisture content was noted slightly low 41.4% and 64.54% for maize seeds as shown in Tables 2 and 4; Figures 2 and 4). Study by Binumol and Santhoshima (2018) reported decrease on seed germination and seedling growth of cowpea due to the allelopathic effect of invasive weed, *Chromolaena odorata*. Malik *et al*. (2022), after conducting a very extensive, reported that among the various studied weeds, *Trianthema portulacastrum* weed was proved more allelopathic in reducing maximum production of maize in Khyber Pakhtunkhwa. Hussain *et al*. (2022) reported the stress caused by *Chenopodium album* in cereals and non-cereals through phytotoxicity, also supported the present findings.

Conclusions and Recommendations

The conclusion drawn from the experiment is that *Verbesina* plant powder have more inhibitory effects on different concentration. All treatments had toxic and inhibitive effects, with methanol extracts having the most notable effects. However, some beneficial effects on growth metrics were also noted. More studies of different experiments are suggested for meaningful conclusion. In residues of 5gm there may be some growth-promoting and bioactive chemicals in hot water extract. To increase agricultural sustainability, environmental safety, food security, resource conservation, and economic stability, this would be a brilliant course to take. To completely investigate the use of *Verbesina encelioides* on adverse effects, more research is required.

It was recommended that by conducting further exploratory studies the plant can be novel potential source for weeds killing.

Author's Contribution

Afsheen Khattak: Research project.
Shahida Naveed: Supervised the whole research process.
Naila Khalid: Helped in data analysis.
Inayat Ullah Khan and Tamana Bakht: Helped in manuscript and data compilation.

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

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