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GU JOURNAL OF PHYTOSCIENCES

GU. J. Phytosci. 1(1): 70-79 (2021)



Bioassay Test of Allelopathic Potential of Sunflower (*Helianthus annuus* L.) against Mung bean (*Vigna radiata* (L.) R. Wilczek)

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Abstract

The Sunflower (*Helianthus annuus* L.) has been well- documented for its high allelopathic potential against different weeds and crop plants. The allelochemicals released by *Helianthus annuus* influenced the germination and growth of other species. Laboratory and greenhouse experiments were conducted to elucidate the allelopathic potential of *H. annuus* against *Vigna radiata*. In this research study laboratory experiments comprised of petri- plate trials which were accomplished by the application sunflower whole plant extracts in different concentrations as 4%, 8% and 12% extract to the germinating seedlings of test crops while the control seedlings were treated with distilled water. Greenhouse experiments were distinguished by whole plant powder was incorporated with soil in different amounts like 4, 8 and 12 g applied to the potted plants, whereas control plants were only filled with soil. The growth parameters like percent germination, germination index, radicle and plumule elongation, seedlings growth, fresh and dry weights and chlorophyll contents of test crop were significantly inhibited by the application of extracts as well as with powder 12 % extract showed greatest inhibition in the growth parameters of test crop. During this research study, the plumule lengths of *V. radiata* were much more affected by the extract concentration as compared to control. Powder extract also contained phytotoxic compounds that caused remarkable reduction in plant heights and chlorophyll contents of test species. It was obvious during this research study that *V. radiata* was resistant against the phytotoxic effects of sunflower. So, it can be concluded from this research observed the allelopathic effects of sunflower extract and powder showed inhibitory effects on different growth parameters which all are essential for plant growth. The main objective of present study is to

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© 2021 (Accepted for publication in June 2021)

Published by Department of Botany, Selection and/or peer-review under supervision of Executive Committee, Ghazi University, DG Khan, 32200 Pakistan

explore the allelopathic effects of sunflower against mung bean, to determine the bioassay test of different plant parameters of test crop and allelopathic effect during plant growth.

Keywords: Allelopathy; Sunflower; Mung bean; Germination

1. Introduction:

The sunflower (*Helianthus annuus* L.) is member of family Asteraceae. It is the most important oil seed crop having high nutritional values. The allelopathic activity of Sunflower has been reported on subsequent crops as well as on weeds (Macais *et al.*, 2002). Sunflower cultivation is done throughout the world as well as in Pakistan as it is a main source of vegetable oil used for different purposes. In Pakistan, it is cultivated during March and April and harvested in June. Sunflowers can be very large and require plenty of space although there are some varieties which have been specially bred to be compact for growing in smaller spaces or for growing in containers (Ali, 1977).

The sunflower has been reported highly allelopathic plant. Most of the members of Asteraceae family are well recognized as allelopathic crop plants on other plants to reduce the germination and seedling emergence of subsequent crops (Bialy *et al.*, 1990; Muehlchen *et al.*, 1990). The allelopathic activity of Sunflower was first time accounted by Leather (1983) and Anjum and Bajwa, (2007). However, Macais *et al.* (2002) and Vyvyan (2002) reported that various phenolic compounds and terpenoids are found in sunflower cultivars. Several phytotoxic allelochemicals that have been identified in sunflower residues are chlorogenic acid, isochlorogenic acid, scopolin and alpha-naphthol derivatives described by Wilson and Rice, (1968). Many other researchers Carter, (1978), Anderson *et al.* (1978), Gawronska *et al.* (2002), Bogatek *et al.* (2006), and Kupidowska *et al.* (2006) also reported the highly allelopathic potential of Sunflower on weeds as well as several crop plants.

The test crop Mung beans (*Vigna radiata* (L.) R. Wilczek) is one of the most important, short-season, summer growing leguminous crop cultivated widely throughout the tropics and subtropics (Thomas *et al.*, 2004). It is native to Asia but widely cultivated in Africa and Latin America (Tomooka *et al.*, 1992; Saravanakumar *et al.*, 2004). It is one of the most important pulse crops of Pakistan that fulfils the nutrition requirement of almost every region. It is mainly cultivated in Southern Punjab and Sindh province during spring season (Ali, 1977). The *V. radiata* is ranked as the second most drought resistant crop after soybean in Asia. It has high nutritional values and has higher protein contents and better digestibility than any other pulse crop (Tabasum *et al.*, 2010). The grains of Mung bean contain 51% carbohydrates, 26% protein, 10% moisture and 3% vitamins. Asaduzzaman, (2008) reported that the remaining of Mung bean are also used as feed for animals as well as enhance the soil fertility. The allelopathic effects of *H. annuus* on several weeds as well as crop plants has been studied. Bashir *et al.* (2012) described that *H. annuus* have allelopathic potential on growth of Rice and Wheat.

1.1. Objectives:

- To explore the allelopathic effects of sunflower against mung bean.
- To determine the bioassay test of different plant parameters of test crop and allelopathic effect during plant growth.

2. Materials and Methods:

2.1. Experimental Design and Collection of Material

The experimental work was conducted in research laboratory and green house of Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus Karachi, Pakistan. The seeds of Sunflower and Mung beans were collected from a local Seed market the purity and germination of these seeds were 90 to 95 %. The field of Sunflower plants was cultivated in the field of Department of Botany, as the plants became young before flowering, the plants were collected.

2.2. Lab Experiment-Preparation of whole plant powder extract

The collected plants were air dried in green house, after drying plants were ground by Wiley mill (Thomas Wiley Lab Mill, Model 4). For the preparation of aqueous extracts, add 4, 8 and 12 g whole plant powder in marked beakers and 50 ml of distilled water was liquescing in each beaker for left the suspension for the time of 24 hours. After 24 hours, these suspensions were centrifuged by centrifuge machine and filtered through Whatman No.1 filter paper and add distilled water to make up the volume up to 100 ml of each concentration and kept the solution into marked conical flasks. All the glass wares were sterilized in autoclave at 121°C and 15 lb pressure for 30 minutes to avoid microbial contamination.

2.3. Petri-Plate Experiment

The seeds of *V. radiata* were surface sterilized with 0.1 % mercuric chloride solution for 1-2 min and washed with distilled water. All the Petri plates were marked as control, 4, 8 and 12 %, along with the 5 replicates, respectively. Total 10 sterilized seeds of both crops were kept in petri-plates along with uniform distance. 2 ml plant powder extracts were poured in the replicates of each concentration while distilled water was poured as a control. The extracts were poured at alternate days. The petri plates of both crops were placed at room temperature in laboratory. The germination of seeds was recorded regularly on daily basis, while the radicle and plumule lengths were taken in alternate days.

2.4. Pot Experiment

Pot experiments were conducted in green house and in an open field of Department of Botany. The sandy loam soil and natural humus fertilizer was used in the ratio of 8:2. The soil was free from fungal population, insects, and other pathogenic organisms. All the pots were marked as control, 4, 8 and 12 g. Total 500 g soil was weighed and incorporated with plant extract powder, while the control pots were filled only with 500 g soil. Pots were kept in green house and irrigated for 2-3 days, and pots were prepared for sowing after 3 days.

2.5. Germination and Plant Growth

The seeds of *V. radiata* was surface sterilized as described previously. Total 10 sterilized seeds were sown in each pot. The germination record was noted on daily basis. After 8 days of germination, the plants were thinned and only four plants were left in each pot. After thinning, the plants height was measured weekly. All the pots were kept in open field due to required critical day light.

2.5.1. Germination Percentage

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2.5.2. Germination Index

The Germination Index was calculated by given formula of Khandakar and Bradbeer, (1983).

$$\text{Germination index (S)} = \left[\frac{N_1}{1} + \frac{N_2}{2} + \frac{N_3}{3} \dots \dots \dots \right] \times 100$$

Where S is the germination index, N1/1, N2/2.... are the ratio of number of the seeds germinated per day

2.5.3. Seedling Vigour Index:

The Seedling Vigour Index (S.V.I.) was calculated according to the following formula of Abdulkaki and Anderson (1973).

$$\text{S. V. I} = (\text{Root length} + \text{Shoot length}) \times \text{Germination (\%)}$$

2.5.4. Inhibition Percentage (%)

Inhibition percentage was calculated by following formula of Surendra and Pota, (1978).

$$\text{Inhibition (\%)} = 100 - \left(\frac{E_2}{E_1} \right) \times 100$$

Where, I= % Inhibition, E1= Response of Control plant, E2=Response of Treatment plant

2.6. Germination and Plant Growth

The pot experiment was terminated after one month and all plants of each pot of experiments were uprooted carefully for taken fresh weight, dry weight, detection of chlorophylls content and carotene.

2.7. Detection of Chlorophyll Contents

2.7.1. Preparation of Samples

For preparation of samples to detection of chlorophyll content, 2g fresh leaves of each concentration (control, 4%, 8% and 12%) were carried out. These leaves were crushed with 2 ml of 80% acetone in pestle and mortar, and then filtrate the extract with the help of muslin cloth. The extract and 2 ml of 80% acetone was centrifuged for 2-3 minutes at 1500 rpm. The supernatant was poured in a separate test tube and debris were washed with 2 ml of 80% acetone. The solution was centrifuged, and the supernatant was transferred into the same test tube. The procedure was repeated

until the debris become colorless. The pestle and mortar were rinsed with 80% acetone and collect the residuals of washing in the same test tube. Make up the volume up to 10 ml with 80% acetone.

2.7.2. Determination of Chlorophyll Content

The chlorophyll content (chlorophyll a, chlorophyll b and carotenoids) was determined by using Spectrophotometer and 80% acetone were poured in the cuvette (at least two-third) it was considered as blank. The Spectrophotometer was kept on constant at 0 absorbance for each wavelength (663 nm, 645 nm, and 510 nm). The blank was used to minimize the amount of 80% acetone in each concentration (control, 4%, 8% and 12%). The O.D of each concentration was carried out and calculated the amount of chlorophyll a, chlorophyll b and total chlorophyll by using the formula given by Arnon, (1949) and content of carotenoids was determined by the formula suggested by Harborne, (1973). Chlorophyll contents can be calculated by the following formula presented by Arnon, (1949).

$$\text{Chlorophyll "a"} \left(\frac{\text{mg}}{\text{g}} \right) = 12.7 (A_{663}) - 2.69 (A_{645}) \times (V|1000) \times W$$

$$\text{Chlorophyll "b"} \left(\frac{\text{mg}}{\text{g}} \right) = 22.9 (A_{645}) - 4.68 (A_{663}) \times (V|1000) \times W$$

$$\text{Total Chlorophyll} \left(\frac{\text{mg}}{\text{g}} \right) = 20.2 (A_{645}) + 8.02 (A_{663}) \times (V|1000) \times W$$

Where, A= Absorbance at specific wavelength, V= Final volume of Chlorophyll extract in 80% acetone, W= Fresh weight of tissue extracted

2.7.3. Determination of Carotenoids

The carotenes content can be calculated by following formula presented by Harborne, (1973).

$$\text{Amount of Carotenes in mg} = \frac{4 \times \text{O. D.} \times \text{Total volume of sample}}{\text{Weight of plant tissues}}$$

3. Results:

3.1. Petri-Plate Experiment

The Petri-Plate experiment was conducted in research lab of Department of Botany. The data of germination percentage was decreased when the concentration of extracts was increased, percent germination began to fall down 100% < 98% < 94% < 94%, respectively. The result of inhibition (%) illustrated that the maximum percent inhibition was observed in 12% extract while minimum percent inhibition was observed in 4% extracts as compared to control. The trend of percent inhibition shown as 0% < 2% < 6% < 6%. The data of germination index expressed that in control treatment the seeds were germinated with greater germination index but when concentrations of extract were increased, the germination index was gradually decreased as 94% < 81% < 63% < 60% (Table 1).

Table 1. Different germination parameters of *Vigna radiata* affected by *Helianthus annuus* in Petri-Plate experiment

Treatment	Different Parameters in Petri-Plate Experiment							
	1	2	3	4	5	6	7	8
Control	100%	94%	0	877.282	8.602±0.206	0	17.082±0.545	0
4%	98%	81%	2	672.69	6.754±0.802	21.48	10.79±0.24	36.78
8%	94%	63%	6	580.83	6.09±0.601	29.2	8.37±0.27	50.96
12%	94%	60%	6	398.614	4.15±0.224	51.7	8.13±0.29	52.35

1= Germination (%), 2= Germination Index, 3= Inhibition (%) in germination (%), 4= S.V.I, 5= Plumule length, 6= Inhibition (%) in plumule length, 7= Radicle length, and 8= Inhibition (%) in radicle length

The observed data recorded on S.V.I. are interpreted that the minimum value was observed in 12% extract as compared to control 4% extract and 8%. The S.V.I. value was decreased as the strength of extracts were increased. This decline trend was obtained as $877.282 < 672.69 < 580.83 < 398.614$. The data of radicle length was observed that the highest reduction was noted in radicle length in 12% extract as compared to control while the lowest reduction was obtained in 4% extract as compared to control. This reduction showed that *H. annuus* inhibit the radicle length as the strength of extracts were increased. The trend was observed as $17.08 < 10.79 < 8.37 < 8.13$ cm. The observations sustained that the maximum rate of inhibition was obtained in 12% extract as compared to control, 4 and 8%. The result showed the trend as $0\% < 36.78\% < 50.96\% < 52.35\%$. The data of plumule length manifested that the length of plumule was significantly decreased as the concentrations of extract were increased. The maximum reduction in plumule length was observed in 12% extract as compared to control, 4 and 8%. The reduction trend showed as $8.60\text{cm} < 6.75\text{cm} < 6.09\text{cm} < 4.15\text{cm}$. The data analysis annotated that the maximum 51.7% percent inhibition was obtained in plumule length in 12% as compared to control (0%) while minimum 21.4% percent inhibition was recognized in 4% and in 8% (Table 1).

3.2. Pot Experiment

The pot experiment was conducted in green house of Department of Botany. The data of germination was observed maximum germination percentage in control while in treatments as the amount of powder was increased, the germination percentage was decreased as in 4, 8, and slightly reduction in 12g powder. The decreasing trend shown as $98 < 92 < 90 < 90\%$. The highest inhibition in germinated seeds was found in 12 g powder extract as compared to control, 4, and 8 g powder extract. The trend of inhibition shown as $0\% < 6.12\% < 8.16\% < 8.16\%$. The data of germination index demonstrated that the germination index was affected as the amount of powder extract increased. The seeds germinated with greatest rate index in control while in 12g powder extract, seeds germinated with lowest rate index. The declined bias shown as $65\% < 62\% < 59\% < 38\%$ (Table 2).

Table 2. Different growth parameters of *Vigna radiata* affected by *Helianthus annuus* in pot experiment.

Treatments	Different Parameters in Pot Experiment						
	1	2	3	4	5	6	7
Control	98%	65%	0%	19.66±0.898	0	5.097±0.57	1.94±0.10
4g	92%	62%	6.12%	17.29±0.94	12.05	4.47±0.24	1.73±0.14
8g	90%	59%	8.16%	16.92±0.42	13.93	4.41±0.28	0.93±0.1
12g	90%	38%	8.16%	15.3±0.678	22.07	4.18±0.60	0.83±0.1

1= Germination (%), 2= Germination Index, 3= Inhibition (%) in germination (%), 4= Plant height, 5= Inhibition (%) in plant height, 6= Fresh weight (g), 7= Dry Weight (g)

The facts substantiated that there was significant decrement scrutinized in heights of 12 g potted plants, while in the other pots as the amount of powder extract increased the plant height slightly decreased in 8 and 4g potted plants as compared to control. The decrement course was obtained as 19.6<17.2<16.9<15.3. The compilations regarded that there is a remarkable inhibition found in the treated plants of 12g powder extract due to greater amount of powder as compared to control plants. The trend shown as 0< 12.0< 13.93<22.07. The fresh weight was found greater 5.09 g in control whereas lowest 4.18g fresh weight was obtained in 12g powder extract. Minimum 0.83 g dry weight was recognized in 12 g powder extract as compared to control 1.94g. The facts and figures demonstrated that there is declined bias archived in fresh weight and dry weight of the treated plants (Table 2).

3.3. Chlorophyll Content:

The data of chlorophyll contents and carotenoids computed in Table 3 illuminated that powder extract in high 12g amount caused reduction of chlorophylls and carotenoids as compared to control.

Table 3. Chlorophyll contents of *Vigna radiata* affected by *Helianthus annuus*

Treatment	Chlorophyll (a) mg/g	Chlorophyll (b) mg/g	Total Chlorophyll mg/g	Carotene
Control	0.046	0.073	0.120	14.2
4g	0.020	0.062	0.08	11.8
8g	0.014	0.045	0.059	8.8
12g	0.0056	0.029	0.035	5.8

4. Discussion

Hussain *et al.* (2004), Hussain and Ilahi, (2009), Samreen *et al.* (2009) stated that allelopathic substances released by plants are incorporated in soil and produced detrimental effects in the fields. Germination of seedling is the first crucial stage for growth and development of the plant. At this stage different metabolic changes take place which is necessary for the growth of the plant. It is indicated from our results that the different strengths of extract caused inhibition in the germination of seedlings of our test crop. There was inhibition in germination was pronounced in the treatments of *V. radiata* when plant extracts of *H. annuus* was incorporated. germination of seeds was reduced at higher concentrations 12% of extract. These results are in accordance with the findings of Kamal and Bano, (2008) who demonstrated that germination of seedlings of wheat was reduced due to aqueous extract of Sunflower. These results also correlated with the results of Khaliq *et al.* (2009) who reported that water extract of Sunflower significantly suppressed the germination of *Cichorium intybus*. The present results also associated with the dissertations of Ashrafi *et al.* (2008) who illustrated that significant inhibition was pronounced in the seed germination of wild barley caused by the application of sunflower extracts. Our results supported with the results of Mubeen *et al.* (2012) who elucidate that sunflower extract has inhibitory effects on germination index of *Dactyloctenium aegyptium*.

Our results supported with the findings of Ahmed *et al.* (1995) who stated that the aqueous or powder extract of *H. annuus* have significant inhibitory effect on germination and seedling growth of Cotton and other plants. In our research studies the radicle and plumule length of *Vigna radiata* was reduced as the extract was applied at greatest strength. Our results justified by the findings of Anderson *et al.* (1978) who documented that radicle and plumule elongation was inhibited due to the whole plant extract of sunflower. Our estimations were not interacting with the determination of Chung and Miller,

(1995) and Turk and Tawaha, (2002) who annotated that radicle growth was much more affected than shoot length due to the extracts of allelopathic plants. Our present results agree with the reports of Brummette and Burns, (1972) reported that phytotoxins present in sunflower residues also inhibit the growth and development of other crops. In present study, fresh weights, and dry weights of treated plants of *V. radiata* decreased as the amount of sunflower powder was associated in higher amount. These results correlate with Anjum and Bajwa, (2005) who demonstrated that the reduction in dry weight of *Phalaris minor*, *Chenopodium album*, *Coronopsis didymus*, *Rumex dentatus* and *Medicago polymorpha* was observed due to the roots, leaves and stem extracts of Sunflower.

Another most important parameter of plant growth is chlorophyll content. Chlorophyll contents are the essential pigments in growth of plants, as they provide basic framework in photosynthesis. The amount of chlorophyll contents destroyed under stress conditions which tremendously affected the metabolic processes of plants. Farhoudi and Lee, (2012) documented that chlorophyll content of *Lolium* spp. as well as *Avena ludoviciana* seedlings reduced due to the application of safflower extract. These results supported our estimations in which the chlorophyll contents were inhibited due to the residues of sunflower. Our results are also in agreement of consistence of Stupnicka-Rodyznkiewicz *et al.* (2006) who elucidate that chlorophyll contents and photosynthetic rate were affected by allelochemicals released by plants residues.

5. Conclusion

H. annuus is an important oil seed crop which is also used in the field of agricultural sciences. In this present research, the allelopathic effect of *H. annuus* were determined on test crop *V. radiata*. It is economically important test crop which is cultivated in large scale. Therefore, it is selected to examine the bioassay investigation of the allelopathic potential of *H. annuus* against *V. radiata*. It has not been previously documented. In our present research, there were differential effects of powder extract of *H. annuus* was distinguished on different growth parameters like percent germination, germination index, radicle and plumule elongation, seedlings height, fresh weight, and dry weight and chlorophyll contents of test crop. During our research study, it was observed that when the sunflower powder extract applied to test crop, the extract showed inhibitory effects on different growth parameters which all were essential for plant growth. It is concluded that sunflower powder extract has allelopathic potential to inhibit the essential growth parameters of cultivated crop.

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