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RESEARCH ARTICLE

Allelopathic Effect of Sunflower on Crops and Pathogens

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ABSTRACT

Helianthus annus L. is a powerful allelopathic plant and contains significant allelochemicals with well-known allelopathic potential against plants. In this research work, the extracts were collected in freshwater, methanolic and desiccated air from seeds and roots. The seedlings of wheat (*Triticum aestivum* L.) and lettuce (*Lactuca sativa*) were treated with those extracts. Our results showed that extracts from the seed and root pose a major effect on the germination of seed, development & arid biomass of wheat and lettuce seedlings. Moreover, the seed extract of *H. annus* affected growth parameters of *T. aestivum* and *L. sativa* than that of root exudates. Similarly, the methanolic extracts of seeds and roots have remarkably reduced root growth in *T. aestivum* and *L. sativa* seedlings. From the data, it is speculated that the allelochemicals of sunflower repressed the wheat and lettuce seedlings' growth, which might have resulted from various cellular processes responsible for reduced plant growth.

Key words: *Helianthus annus* L., wheat, allelochemicals

INTRODUCTION

The word allelopathy is derived from the Greek word `allelon' meaning `each other' and `pathos' meaning `suffering' and was presented by Hans Molisch. Allelopathic chemicals are present in different parts of a plant (i.e. roots, stems, leaves, fruits and flowers). These chemicals are poisonous and may retard seed germination or growth of the stem, root. Allelochemicals are synthesized in the stems and leaves, which are released by a process of exudation from roots into the environment or breakdown of plant resources (Jilani et al., 2008). These different types of phytotoxins inhibit the germination and development of unlike crops in surrounding areas (Batish et al., 2004). *Helianthus annus* L. (sunflower) a member of the family Asteraceae, contains a stimulated amount of bioactive allelochemicals that have great harmful effects on other plant organs, tissues and cells (Galisteo et al., 2023). The sunflower has a strong allelopathic activity that can even inhibit the germination of its own seeds (Azania et al., 2003). The most important allelochemicals of sunflower are phenol, flavonoids and terpenoids (Rawat et al., 2017). Somewhere sunflower is planted, it expresses an allelopathic movement within the soil (Grodzinskii, 2016). Many of the allelochemicals have been reported from the sunflower that pass from one to another through biological activity. In soils where herbicides are restricted, sunflower allelopathic activity is used to control weeds in that condition such as organic farming. Sunflowers can aggressively power the intensification of nearby shrubbery's elevated allelopathy (Qasem and Foy, 2001). More than two

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hundred natural allelopathic compounds have been isolated from different cultivars of sunflower (Nikneshan et al., 2011). The leaching of these compounds to the surroundings results in the reduction of germination and expansion of other species, which might change the function and structure of nearby plants in a few days. Generally, allelochemicals are spread through rain leaching, remaining components, or exudates (Ashrafi et al., 2008). These exudates or leachates showed inhibitory or stimulatory effects depending on the nature and characteristics of released allelochemicals (Scavo et al., 2019).

Helianthus annus L. is an annual plant, with a height of 1.5-2.5 m and a single apical inflorescence (Waldah, 2018). The flower has a diameter of 15-30 cm which consists of frequently yellow and sterilized ligulae or ray flowers and the productive disc or tube flowers (Elhag, 2017). *H. annus* L. is one of the most significant oilseed crops in the world as the plant is an inhabitant of the USA and Mexico. It was introduced in Europe in the 16"'century, initially in Spain and later in England and France (Dharmraj et al., 1994). Sunflower is not only used as an oil seed crop (Park et al., 1992) but the seeds are also a source for making flour, trimmings for a few dinner services, or being addicted to roast. The plant is heat and light-sensitive, which are cultivated around the year in tropical & sub-tropical areas of the world. Sunflowers can be grown in every soil, but the best is a well-drained, high water-holding capability soil with a neutral pH (6.5-7.5). The poor growth of sunflower plants in winter and summer is not due to mineral deficiency in the soil but the remaining allelochemicals present in the soil (Pariana, 1992).

Allelochemicals are less important plant metabolites. This process involves the release of chemicals into the ecosystem. These chemicals have a detrimental effect on crops in the ecosystem, leading to reduced and delayed germination, seedling death, growth and yield reduction (Alsaadawi et al., 2012). *H. annus* is a high-quality vegetable crop of oil that is not strictly weather-limited and can be planted twice a year in Pakistan. As a successful allelopathy, its effects on succeeding crops and weeds have been reported (Cheema et al., 2013). *Triticum aestivum* is a very important cash crop in Pakistan with a very low yield per hectare. Lower yield per hectare may be due to poor germination and residual effects of adjoining allelopathic plants (Duke, 1985).

Objectives of Research

- To investigate the allelopathic effects of *H. annus* seed coat exudation on *L. sativa* and *T. aestivum* seeds.
- To analyse the allelopathic effects of exudates of the root of sunflower on lettuce and wheat seeds.
- To analyse any potential effect on pathogens (as antibacterial).

MATERIALS AND METHODS

Plant Selection

The sunflower plant was selected for allelopathic effects, while the experiment was performed at the Laboratory of Botany Department. Abdul Wali Khan University Mardan.

Extraction and Testing of Sunflower Seed-Coat Exudates

Twenty seeds were taken in 15 ml water in a falcon tube and kept in a shaking incubator for three days at 25 °C. The resultant extract was collected in falcon tubes and freeze-dried. The frozen-dried exudates were redissolved in ddH_2O and applied to the lettuce seeds.

Extraction and Testing of Sunflower Root Exudates

Sunflower seeds were sown on autoclaved sands and were kept in the light. The growth started after a few days. The seedlings were transplanted into 25 glass tubes. Each glass tube contained four seedlings of sunflower. These glasses were kept under the light for 12 days. By examining after 12 days the plants became stronger and larger than earlier and received more exudates with the help of roots. The plants were removed and exudates of root were stored in bottles. The root exudates were tested against the lettuce plant.

Study of Allelochemicals

The effects of allelopathy of seed extract as well as root exudates of *H. annus* were tested on wheat and lettuce.

Anti-Microbial Activity

The antimicrobial activities were performed through the well diffusion method. Human pathogenic bacteria (available at the University) were treated with seed and root extracts. We used all these pathogenic bacteria against the growth of sunflower exudates.

Apparatus Sterilization

Dissimilar types of apparatus or Petri plates were used for dissimilar experiments in the research work like antibacterial activities and allelopathy of *H. annus*. These all apparatus were sterilized through an autoclave at 120 °C & specific pressure.

Microscopic Analyses

Microscopic analysis was performed as per the requirements of our experiment.

RESULTS

Germination Percentage of Seeds

Seed germination is the process by which new plants emerge from the seeds under positive conditions which include sunlight, oxygen, water and air temperature. The mean of seed germination was taken. The highest germination of the seed was observed in control of both lettuce and wheat plants, while the lowest germination rate of seed is recorded frequently within root exudates and seed extract. The subsequent table shows the germination of lettuce and wheat.

Table 1.1: Germination percentage of lettuce seeds

H.annus Extract	Number of seeds	Seeds germinated	Germination (%)
Control	30	29	96.66%
Root exudates	30	29	96.66%
Seed extract	30	2	6.66%

Table 1.2: Germination percentage of wheat seeds

H.annus Extract	Number of seeds	Seeds germinated	Germination (%)
Control	30	25	83.33%
Root exudates	30	20	66.66%
Seed extract	30	15	50%

LETTUCE

Shoot and Root Length

The shoot and root length were measured after exposure to seedling exudates and seed extract. The shoot and root length were greatly affected by the bioactive compounds present in seedling exudates and seed extract. The highest growth was observed in the control plants and the lowest growth was observed in the plants exposed to seed extract (Fig. 1.1). The root shoot fresh length of lettuce is taken in centimeters.

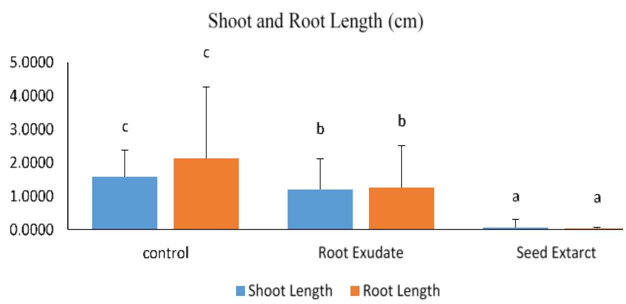

Fig. 1.1: Effect of a) seed extract and b) root exudates of *H. annus* on the root shoot length of Lettuce plants. Data are the means of three replicates with standard error bars. Different letter shows significant differences ($p < 0.05$).

Shoot Fresh Weight and Root Fresh Weight

The SFW and RFW were measured after exposure to seedling exudates and seed extract. The shoot and root length were greatly affected by the bioactive compounds present in seedling exudates and seed extract. The highest growth was observed in the control plants and the lowest growth was observed in the plants exposed to seed extract (Fig. 1.2). The shoot root fresh weight of lettuce is taken in grams.

Shoot Dry Weight and Root Dry Weight (SDW+RDW)

The shoot and root dry weights were calculated after exposure to seedling exudates and seed extract. The shoot root length was greatly affected by the bioactive compounds present in seedling exudates and seed extract. The highest growth was observed in the root exudate plants and the lowest growth was shown in the plant's exposure to seed extract (Fig. 1.3). The root shoot dry weight of lettuce is taken in grams.

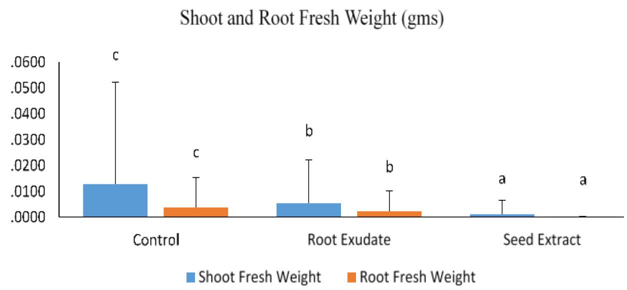

Fig. 1.2: Effect of a) seed extracts b) root exudates of *H. annus* on the shoot and root fresh weight of Lettuce plants. Data are the means of three replicates with standard error bars. Different letter shows significant differences ($p<0.05$).

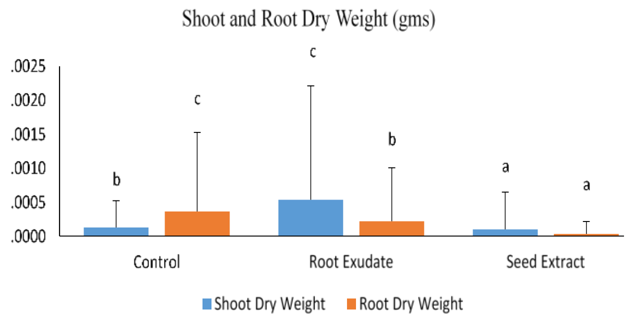

Fig. 1.3: Effect of a) seed extract b) root exudates of *H. annus* on the shoot root dry weight of Lettuce plants. Data are the means of three replicates with standard error bars. Different letter shows significant differences ($p<0.05$).

WHEAT

Shoot and Root Length

The root shoot lengths of wheat were measured after their exposure to the seedling exudates and extract of seeds of sunflower. The root shoot lengths of wheat were greatly affected by the bioactive compounds present in the extract of sunflower seeds and seedling exudates. The highest growth was noted in the control plants whereas the lowest growth was recorded in the plants exposed to seed extracts (Fig. 1.4). The root shoot length of wheat is taken in centimeters.

Shoot and Root Fresh Weight

The shoot and root fresh weight of wheat were recorded after exposure to seedling exudates and seed extract of sunflower. The shoot fresh weight and root fresh weight were greatly affected by the bioactive compounds of seedling exudates and seed extract. The highest growth was recorded in the control and the lowest was observed in the plants exposed to the seed extract (Fig. 1.5). The shoot root fresh weight of wheat is taken in grams.

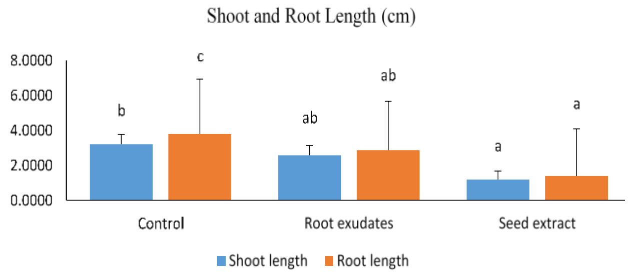

Fig. 1.4: Effect of different treatments a) seed extract b) root exudates of sunflower on the root shoot length of Wheat plants. Data are the means of three replicates with standard error bars. Different letter shows significant differences ($p < 0.05$).

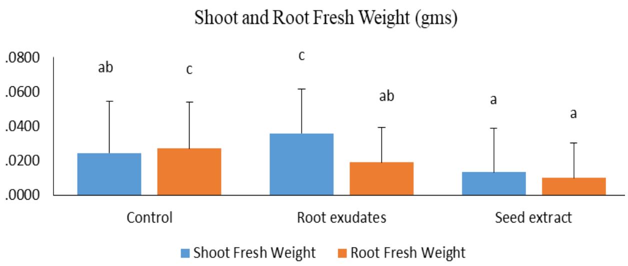

Fig. 1.5: Effect of different treatments a) seed extract ab) root exudates of sunflower on the root shoot fresh weight of Wheat plants. Data are the means of three replicates with standard error bars. Different letter shows significant differences ($p < 0.05$).

Shoot Dry Weight and Root Dry Weight

The shoot dry weight and root dry weight of wheat were measured after exposure to seedling exudates and seed extract. The shoot dry weight and root dry weight were greatly affected by the bioactive compounds of seedling exudates and seed extracts. The highest growth was recorded in control plants, while the lowest growth was shown in the plants exposed to seed extract (Fig. 1.6). The root shoot dry weight of wheat is taken in grams.

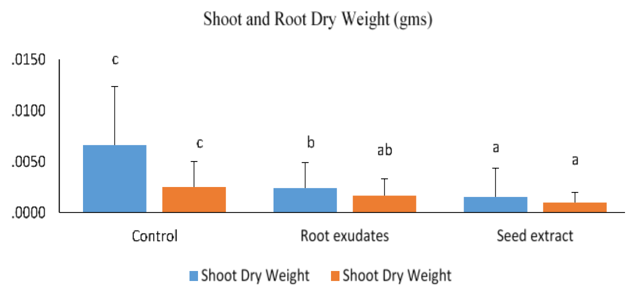

Fig. 1.6: Effect of different treatments a) seed extract b) root exudates of sunflower on the root shoot dry weight of Wheat plants. Data are the means of three replicates with standard error bars. Different letter shows significant differences ($p < 0.05$).

Anti-Microbial Activity

The antibacterial activity of *Helianthus annus* has been performed by agar well diffusion method. For bacterial growth, the LB agar media was used. Seed extract and root exudates were used to confirm the antibacterial activity. During analysis of antibacterial activity, there was no such activity detected in seed extract and root exudates of *H. annus*. Antibacterial activity was performed on seed extract and root exudates of *H. annus.* A standard antibiotic used in this activity was streptomycin (2 mg/ml) as a positive control, while ddH_2O was a negative control in this activity.

Staphylococcus aureusis

*Staphylococcus aureus*is resistant to seed extract and root exudates. The negative control is ddH_2O while the positive control streptomycin can show resistance to *Staphylococcus aureus* and create an inhibitory zone that is larger than 8 mm. This showed that *H. annus* has no antibacterial activity.

Pseudomonas aeruginosa

*Pseudomonas aeruginosa*is also resistant to seed extract of *H. annus* and there was no inhibitory zone in root exudates and seed extract. Streptomycin was used as the positive control and showed resistance to *Pseudomonas aeruginosa*, while the negative control was ddH_2O.

Escherichia coli

There was no effect of seed extract and seed exudates of *sunflower* on *E. coli.* Streptomycin and ddH_2O were used as controls.

Klebsiella pneumonia

Klebsiella pneumoniae is highly resistant to seed extract and root exudates and there was no inhibitory zone against *Klebsiella pneumoniae,* which shows that it is resistant to the seed extract and root exudates of *H. annus.* The positive control was Streptomycin, and the negative was ddH_2O.

Clavibacter michiganensis

The seed extract and root exudates of *H. annus* were applied on *Clavibacter michiganensis,* and there was no effect of seed extracts and root exudates. The positive control was Streptomycin and the negative was ddH_2O.

Morganella morganii

There was no effect of seed extract and root exudate on *Morganella morganii.* Only positive control streptomycin can make a visible zone of inhibition and resistant to *Morganella morganii.* Negative control ddH_2O has also no effect on the *Morganella morganii.*

DISCUSSION

Sunflower is an allelopathic plant with many plant toxins that have known allelopathic effects on weeds and a lot of other crops. This experiment opened a strong allelopathic effect of *H. annus* on lettuce and wheat germination and seedling growth parameters (Hamad 2017). Typically, the seed extract has a relatively higher phytotoxicity than the further parts of the sunflower used in this study. Sunflower has many allelochemicals, mainly flavonoids, alkaloids, terpenoids and phenolic compounds, which have a complete allelopathic effect on several plants. These chemicals are derived metabolites that participate in the modification of adjacent flora and microorganisms through inhibition or stimulation. Allelochemicals habitually result in germination hang-up and later in competing plants, condensed free radical and germ growth, and reduced dry substance content. Therefore, the reduced germination rate, germination index, average germination time delay and reduced root and stem growth of wheat and lettuce seedlings in this learning may be credited to possible allelochemicals in sunflowers (Cheng and Cheng 2015). Mutation in cell division, normal function and absorptive ability of given plant seedlings were affected (Volkenburgh 1999). The allelopathic activity of dissimilar parts of sunflower (roots, branches, seeds and seedlings) on *T. aestivum* and lettuce may be owing to various classes of elements present in these tissues (Soltys et al., 2013). Moreover, extraction from fresh sunflower seeds, seedlings and root exudates greatly inhibited the germination of wheat and lettuce seeds and seedlings, when compared to the control. The germination rate was reduced by 20% to 50%. As the absorption of the seed extract amplified, the degree of inhibition of the root and shoot increased.

Conclusion

Current research provides evidence that the *H.annus* has strong allelopathic activity. Further, the results revealed that sunflowers drastically abridged the germination and expansion of the wheat as well as the lettuce plant. The findings verified that under laboratory conditions, sunflower is one of the best allelopathic species that releases some allelopathic compounds, including alkaloids, flavonoids, tannin, etc. that can retard the germination and growth of the receiver species. The allelochemicals present in sunflower exudates confirmed small and extensive time-destructive allelopathic effects on lettuce and wheat that can be used as potential weedicides or herbicides in the future.

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