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Original Article

Allelopathic Effects of sunflower (*Helianthus annuus*) on Germination and Growth of Seedling Cow Soapwort (*Vaccaria hispanica*) & Russian knapweed (*Acroptilon repens*)

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Abstract

Sunflower [*Helianthus annuus* (L.) Koch.] contains watersoluble allelochemicals that inhibit the germination and growth of other species. This characteristic could be used in weed management programmers'. Greenhouse and laboratory experiments were conducted to determine the effects on *Vaccaria hispanica* and *Acroptilon repens*. Its effect on two weedy species *V. hispanica* and *A. repens* was studied with a view to explore its herbicidal potential. Germination of both the weeds was reduced with increasing concentration of sunflower and a dose-response relationship was observed. This provided information on LC50 and Inhibition threshold concentrations of sunflower that could be useful for future studies. Further, sunflower also inhibited the growth of both the weeds in terms of root and shoot length and seedling dry weight. Inhibition of root growth was greater than that of shoot growth. Similar observations were made when the test weeds were grown in soil amended with different concentrations of sunflower. In addition to growth, there was a reduction of chlorophyll content in the growing seedlings. It also caused water loss in the weedy species. The study, therefore, reveals that sunflower exerts an inhibitory effect on the growth and development of both weeds and can be further explored as an herbicide for future weed management strategies.

Keywords: *Allelochemical; Herbicidal activity; Dose–response relationship; Seedling growth; Sesquiterpene lactone*

INTRODUCTION

Modern agriculture is productivity oriented and thus relies heavily on the use of synthetic chemicals to control weeds and other pests. This has undoubtedly enhanced crop production but at the same time may have a negative impact on the environment quality and on human health. Further, the development of resistance among weeds to synthetic herbicides is also a cause for concern (1, 2, 3,5). Due to the repercussions associated with the use of synthetic chemicals, it becomes desirable to find new classes of compounds with novel sites of action. Natural plant products that are biodegradable, exhibit structural diversity and complexity, and rarely contain halogenated atoms constitute one such class of chemicals (1,4,5,6,7,9). These can act directly as herbicides or may provide lead structures for herbicidal discovery (8,10). Besides, they tend to act on unexploited target sites (11). In order to identify plants with biologically active natural products, selection of allelopathic plants is a good and commonly used approach (4,6,10) Allelopathic properties of this weed have been well demonstrated (11, 12, 13). These are largely attributed to the presence of sunflower—a sesquiterpene lactone of pseudoguanolide nature found in various parts of the weed (14,15,16,18). It is found sequestered in trichomes that cover the whole plant and this probably prevents its interference with its own vital physiological processes (17,18). Sunflower is known to be phytotoxic against many plants including aquatic ones (5,6,2,19,20,22,24). However, its phytotoxic nature has not been exploited for weed and pest management, though a little work has been done in this direction (5,6,23,24,26). Since the use of

natural plant products, particularly the allelochemicals, for the management of noxious weeds is a logical strategy (1,5,12,), we hypothesized that sunflower could be a potential natural herbicide, if screened. Keeping this in mind, we studied the effect of sunflower against two weed species viz. *A.repens* and *V. hispanica* with a view to explore its herbicidal potential.

MATERIALS AND METHODS

Plant material

Leaves of *H. annuus* (sunflower) were collected locally from wild growing stands. These were shade-dried and powdered. Seeds of *Acroptilon repens* (Russian knapweed) were obtained from Department of Agronomy, Agricultural Paradise, University of Tehran whereas those of *V. hispanica* (Cow Soapwort) were collected locally from the plants growing in and around the Research farm, Department of Agronomy, Agricultural Paradise, University of Tehran.

Extraction of sunflower

Sunflower was extracted from the shade-dried leaves of *H. annuus* following the method of Saxena et al. (27). For growth experiments, solutions of sunflower were prepared by dissolving the requisite amount of sunflower in 2 ml of absolute alcohol and made the final volume with distilled water.

Dose–response studies

Effects of different concentrations of sunflower i.e. 38, 76, 152, 228, 304, 380, 475, 570, 665, 760, 950, 1140, 1520 and 1900 µM were studied on the germination of *V. hispanica* and *A.repens* in a laboratory bioassay. Fifty seeds of *V. hispanica* and twenty of *A.repens* were germinated in a 15 cm diameter Petri dish lined with a Whatman no. 1 filter paper moistened with the respective treatment concentration of sunflower solution. Treatment with distilled water instead of sunflower solution in a similar way served as control. For each treatment there were five replications. Petri dishes were placed in a growth chamber at 25±2 °C temperature, a 16-h light: 8-h dark photoperiod, photon flux density of approximately 150 µmol m^{-2} s^{-1} and relative humidity of around 75%. After a week, the numbers of seeds that germinated was counted.

Growth experiments

In another set of experiments, the effect of four concentrations of sunflower i.e. 190, 380, 570 and 760 _M, selected on the basis of dose-response studies, was studied on the early growth of *A.repens and V. hispanica*. Fifty seeds of *V. hispanica* and twenty of *A.repens* were allowed to germinate and grow in a 15 cm diameter Petri dish lined with a Whatman no. 1 filter paper moistened with the respective sunflower solutions. For each treatment there were three replicates. Treatment with distilled water in a similar manner served as control. The entire set-up was kept in a growth chamber at 25±2 °C temperature, 73±2% relative humidity, and 16-h light: 8-h dark photoperiod and photon flux density of approximately 150 µmol photons m^{-2} s^{-1}. After 10 days, seedling growth (in terms of radical length and shoot growth), and seedling dry weight were measured. The experiment was repeated twice. Based on the growth experiments, LC50 concentration (the lowest concentration at which 50% germination occurs) was determined.

Effect of sunflower mixed in soil

For this experiment, garden soil was collected from free area, air-dried and sieved through a 2 mm sieve. Soil was sandy loam in nature (sand 51%, silt 27% and clay 22%) with pH 7.15, conductivity 0.06 µmhos cm^{-1}, organic carbon 0.88% and water holding capacity 41.8%. An amount of 310 g of soil was treated with 100 ml each of 190, 380, 570 and 760 µM of sunflower or distilled water to serve as a control. The treated soils were left as such for 8 h. It was then seeded with fifteen seeds of *A.repens* a 30 seeds of *V. hispanica*. Three replicates were maintained for each treatment. The entire-set up kept at 16-h light: 8-h dark cycle of approximately 150 µmol photons $m^{-2}s^{-1}$. Every other day after sowing, all the treated soils received 50 ml of each of the respective sunflower solution whereas 50 ml of distilled water was added to control soil. After 2 weeks, root and shoot length, dry weight, chlorophyll content and water content of the emerged seedlings were measured.

Chlorophyll content

Chlorophyll was extracted using dimethyl sulphoxide following the method of Hiscox and Israelstam (1979) and estimated using the equation of Arnon (1949). It was expressed in terms of dry weight of the tissue as suggested by Daizy and Kohli (1991).

Statistical analysis

Percent changes in germination, root and shoot length, chlorophyll content and water content were subjected to one-way analysis of variance (ANOVA) followed by separation of means at 0.05% level applying the multiple range test.

RESULTS

Sunflower exerted a phytotoxic influence on the germination of both the weedy species, i.e. *A.repens* and *V. hispanica* as depicted by the dose–response curve. The germination of the test weeds was measured to be less at all the concentrations of sunflower and the decrease continued with the increasing concentration exhibiting a strong reciprocal correlation. From the dose–response curve, it is also clear that the inhibition threshold began right from 38 µM (the lowest concentration used) of sunflower and onwards. The LC50 concentrations were calculated to be approximately 650 and 720 µM, respectively, for *A.repens* and *V. hispanica*.

In addition to germination, even the subsequent growth measured in terms of root and shoot length and seedling dry weight, was drastically reduced at 190, 380, 570 and 760 µM concentrations of sunflower (Table 1). The inhibitory effect was greater on the growth of roots than shoot length. In response to the 190 µM of sunflower the lowest concentration used for growth experiments, root length was reduced by nearly 71% in *V. hispanica* and 51% in *A.repens*. At 760 µM—the highest concentration of sunflower tested the root length was reduced by over 93% in both the weed species (Table 1).

Table 1: Effect of sunflower extract on root and growth and dry weight of *Vaccaria hispanica* and *Acroptilon repens*

Concertration (µM)	Root length(cm)		Shoot length(cm)		Dry weight (mg per plant)	
	V. hispanica	*A. repens*	*V. hispanica*	*A. repens*	*V. hispanica*	*A. repens*
0(Control)	6.3 a	4.2 a	5.0 a	3.65 a	14.2 a	11.3 a
190	3.9 b	1.01 b	3.4 b	1.8 b	11.6 b	7.8 b
380	2.3 c	0.66 c	1.8 c	0.88 d	6.33 c	3.2 c
570	1.8 d	0.42 d	0.77 d	0.71 d	4.01 d	2.9 c
760	0.74 e	0.27 e	0.29 e	0.18 c	2.65 e	1.7 d

Data are mean (±S.D.) of their replicates: different superscripts in a column indicate difference at $P < 0.05$

The inhibitory effect was greater on the growth of roots than shoot length. In response to the 190 µM of sunflower the lowest concentration used for growth experiments, root length was reduced by nearly 71% in *V. hispanica* and 51% in *A.repens*. At 760 µM—the highest concentration of sunflower tested the root length was reduced by over 93% in both the weed species (Table 1).

Almost similar trend of changes were observed in case of shoot length and dry weight. Between the two weedy species, more inhibitory effect was observed on the *V. hispanica* compared with *A.repens* (Table 1). Keeping in mind the phytotoxic effects of sunflower on the germination and growth of the weeds tested, studies were extended to monitor the effect of sunflower added to soil on the initial growth of both the weeds. It was observed that sunflower exerted a strong inhibitory effect on root and shoot length and dry weight accumulation in 2-week-old seedlings of both weed species (Table 2).

Table 2: Growth performance of *Vaccaria hispanica* and *Acroptilon repens* grown in soil

Concertration(µM)	Root length(cm)		Shoot length(cm)		Dry weight (mg per plant)	
	V. hispanica	*A. repens*	*V. hispanica*	*A. repens*	*V. hispanica*	*A. repens*
0(Control)	9.3 a	6.4 a	6.2 a	4.65 a	13.6 a	12.3 a
190	5.9 b	3.4 b	4.1 b	2.2 b	10.1 b	8.6 b
380	4.1 c	1.7 c	2.8 c	1.4 d	7.3 c	4.2 c
570	3.8 c	0.82 d	1.6 d	0.95 d	4.33 d	2.7 c
760	1.7 d	0.3 e	0.63 e	0.38 c	2.45 e	1.5 d

Data are mean (±S.D.) of their replicates: different superscripts in a column indicate difference at $P < 0.05$

In this experiment also, root length was more affected compared with shoot length. At 190 µM of sunflower added to soil, root length was reduced by nearly 42 and 41% in *V. hispanica* and *A.repens*, respectively, whereas at 760µM, it was reduced by nearly 71% in both the weedy species

(Table 2). Here also, more inhibitory effects were observed on *V. hispanica* compared with *A.repens*. Furthermore, the chlorophyll content of seedlings growing in sunflower treated soil was also decreased thereby probably affecting the photosynthetic activity. It was observed to be significantly less in response to all the concentrations of sunflower and the effect was more prominent in *A.repens* (Table 3). Not only the chlorophyll content, even the content of water in treated seedlings was reduced indicating water loss in response to sunflower. More water loss was observed in case of *V. hispanica* compared with *A.repens* (Table 3).

Table 3: Amount of total chlorophyll and water content in 2-week-old seeding of *Vaccaria hispanica* and *Acroptilon repens* grown in soil amended with sunflower

Treatment (μM)	Total chlorophyll (mg g^{-1})		Water content (%)	
	V. hispanica	*A. repens*	*V. hispanica*	*A. repens*
0(Control)	5.05 a	3.4 a	46.2 a	44.65 a
190	4.7 b	2.1 b	40.1 b	39.2 ab
380	4.1 c	1.2 c	32.8 bc	34.4 d
570	4.28 c	0.82 d	24.6 c	28.6 c
760	2.7 d	0.3 e	17.63 d	23.1 d

Data are mean (±S.D.) of their replicates: different superscripts in a column indicate difference at $P < 0.05$

Discussion

It is clear from the present study that sunflower exhibit an inhibitory effect on the germination and growth of both the test weed species - *A.repens* and *V. hispanica*. From the dose-response studies LC50 concentrations were determined for both the species. Determination of these values bears a great significance as they may serve as frames of reference for subsequent studies and can be useful in determining both qualitative and quantitative influence in evaluating the effect of an inhibitor (6,8,10). Further the growth of test weeds was also reduced with the treatment of sunflower. Although the reasons for impaired growth could not be determined in these experiments, it can be pointed out that sunflower might be acting through modes already reported. Sunflower (in general sesquiterpene lactones) react with _SH group of amino acids and proteins and modify their original properties (11,13). Pandey (22) reported that inhibition of growth of water hyacinth by sunflower is due to the various physiological changes brought about by damage of cellular membrane and loss of dehydrogenase activity in roots and loss of chlorophyll in leaves. Batish et al. (6,7,9) have demonstrated that sunflower impairs mung bean growth by affecting respiration, protein content and activities of protease and peroxidase enzymes. From the study, it is also clear that sunflower exerted more effect on root than on shoot. Such an observation that sunflower is a potent root inhibitor has also been made earlier by Kohli et al (11,13) and Hiscox, andIsraelstam (15,17). The growth inhibitions were also monitored in the soil treated with sunflower solutions of different concentrations. In this case not only growth, even the chlorophyll content and Water content was also reduced in the seedlings growing in treated soil compared with untreated control. The reduction in chlorophyll content in response to allelochemicals has been reported in a number of plants (4,8,13,14,22,23,25). However, it is not clear whether the observed loss in chlorophyll was due to degradation of chlorophyll already present in the plant or to direct inhibition of chlorophyll biosynthesis. Nevertheless, the loss of chlorophyll is likely to reduce the photosynthetic ability and thereby the growth and development of the plant. Further, the decrease in water content indicates water loss due to sunflower treatment. This observation is in conformity with that of Pandey (22) who reported that sunflower causes water loss due to root dysfunction. Thus, sunflower causes considerable toxicity to test weeds in soil too. However, much needs to be done in this direction as regards its fate and dynamics in soil. As regards the biodegradation of the sunflower in the soil, no study is available. However, in aquatic environment the phytotoxicity of sunflower is gradually lost in about 30 days under outdoor conditions making lethal dose unlethal (6,8,20,22).

The present investigation that was aimed at determining primary and secondary screens for sunflower indicates that sunflower is a promising phytotoxin with a potential for development of natural product herbicide. However, its interaction with other biotic and abiotic factors such as nutrients and microorganisms, characterization of its post- or pre-emergent herbicidal activity and

mode of action needs to be explored. As regards the crop selectivity of the sunflower, very little is known. There has been a preliminary study reporting that sunflower is selective in action against bill-goat weed (*Ageratum conyzoides*) and wheat. The concentrations at which the germination and growth of bill-goat weed were inhibited/ reduced; there was no effect on the germination of wheat although a marginal effect on the growth was observed (9,12,14,23,25). From the present study, it could be concluded that sunflower possesses weed suppressing ability that can be utilized for future weed management strategies.

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