

Allelopathic Potential of Sunflower (*Helianthus annuus*) against Seed Germination in Wild Mustard (*Sinapis arvensis*) and Foxtail (*Setaria viridis*)

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ABSTRACT

Greenhouse and laboratory experiments were conducted to determine the allelopathic effects of sunflower on *Sinapis arvensis* and *Setaria viridis* with a view to explore its weed seed inhibition potential. Germination of both the weeds was reduced with increasing concentration of sunflower extract and a dose-response relationship was observed. These curves provided information on LC₅₀ and inhibition threshold concentrations of sunflower extracts. 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 extract. Reduction of chlorophyll content and water loss in the growing seedlings was also observed. The study, therefore, revealed that sunflower exerted an inhibitory effect on the growth and development of both the weeds and can be further explored in future for weed management strategies.

Key words : Sunflower, allelopathy, 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. 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 (Einhellig and Rasmussen, 1979; Duke *et al.*, 1997; Dayan *et al.*, 1999; Economou *et al.*, 2002; Ashrafi *et al.*, 2007). These can act directly as herbicides or may provide lead structures for herbicidal discovery (Einhellig and Rasmussen, 1979; Economou *et al.*, 2002). Besides, they tend to act on unexploited target sites (Economou *et al.*, 2002). In order to identify plants with biologically active natural products, selection of allelopathic plants

is a good and commonly used approach (Economou *et al.*, 2002; Ashrafi *et al.*, 2007) Sunflower is known to be phytotoxic against many plants including aquatic ones (Picman and Picman, 1984; Hall and Henderlong, 1989; Batish *et al.*, 1997; Azania *et al.*, 2003; Ashrafi *et al.*, 2007). However, its phytotoxic nature has not been exploited for weed and pest management, though a little work has been done in this direction (Whittaker and Feeny, 1977; Turk and Tawaha, 2002; Azania *et al.*, 2003). Since the use of natural plant products, particularly the allelochemicals, for the management of noxious weeds is a logical strategy (Duke *et al.*, 2000), we studied the effect of sunflower against two weed species viz., *S. viridis* and *S. arvensis* with a view to explore its allelopathic 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 *S. viridis* were

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obtained from Department of Agronomy, Indian Agricultural Research Institute, whereas those of *S. arvensis* L. (Wild mustard) were collected locally from the plants growing in and around the Panjab University campus.

Extraction of Sunflower

Sunflower was extracted from the shade-dried leaves of *H. annuus* following the method of Saxena *et al.* (1991). 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 µg/l were studied on the germination of *S. arvensis* and *S. viridis* in a laboratory bioassay. Fifty seeds of *S. arvensis* and 20 of *S. viridis* 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/m²/s and relative humidity of around 75%. After a week, the number 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 µg/l, selected on the basis of dose-response studies, was studied on the early growth of *S. viridis* and *S. arvensis*. Fifty seeds of *S. arvensis* and 20 of *S. viridis* 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, 16-h light : 8-h dark photoperiod and photon flux density of

approximately 150 µmol photons/m²/s. After 10 days, seedling growth (in terms of radicle 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 52%, silt 28% and clay 20%) with pH 7.05, conductivity 0.04 µmhos/cm, organic carbon 0.93% and water holding capacity 41.2%. An amount of 300 g of soil was treated with 100 ml each of 190, 380, 570 and 760 µg/l 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 15 seeds of *S. viridis* or 30 seeds of *S. arvensis*. 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/m²/s. 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 two 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 Batish *et al.* (2002). It was expressed in terms of dry weight of the tissue as suggested by Daizy and Kohli (1991).

Statistical Analysis

Per cent 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 AND DISCUSSION

Sunflower exerted a phytotoxic influence on the germination of both the weedy species i. e. *S. viridis*

and *S. arvensis* as depicted by the dose-response curve (Fig. 1). The germination of the test weeds was measured to be lower at all the concentrations of sunflower (relative to control) and the decrease continued with the increasing concentration exhibiting a strong reciprocal correlation (Fig. 1). From the dose-response curve, it is also clear that the inhibition threshold began right from 38 $\mu\text{g/l}$ (the lowest concentration used) of sunflower and onwards. The LC50 concentrations were calculated to be approximately 625 and 700 $\mu\text{g/l}$, respectively, for *S. viridis* and *S. arvensis* (Fig. 1). 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 $\mu\text{g/l}$

concentrations of sunflower (Table 1). The inhibitory effect was greater on the growth of roots than shoot length. In response to the 190 $\mu\text{g/l}$ of sunflower the lowest concentration used for growth experiments, root length was reduced by nearly 74% in *S. arvensis* and 49% in *S. viridis*. At 760 $\mu\text{g/l}$ —the highest concentration of sunflower tested the root length was reduced by 88 and 93% in *S. arvensis* and *S. viridis*, respectively (Table 1). Almost similar trend of changes was observed in case of shoot length and dry weight. 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

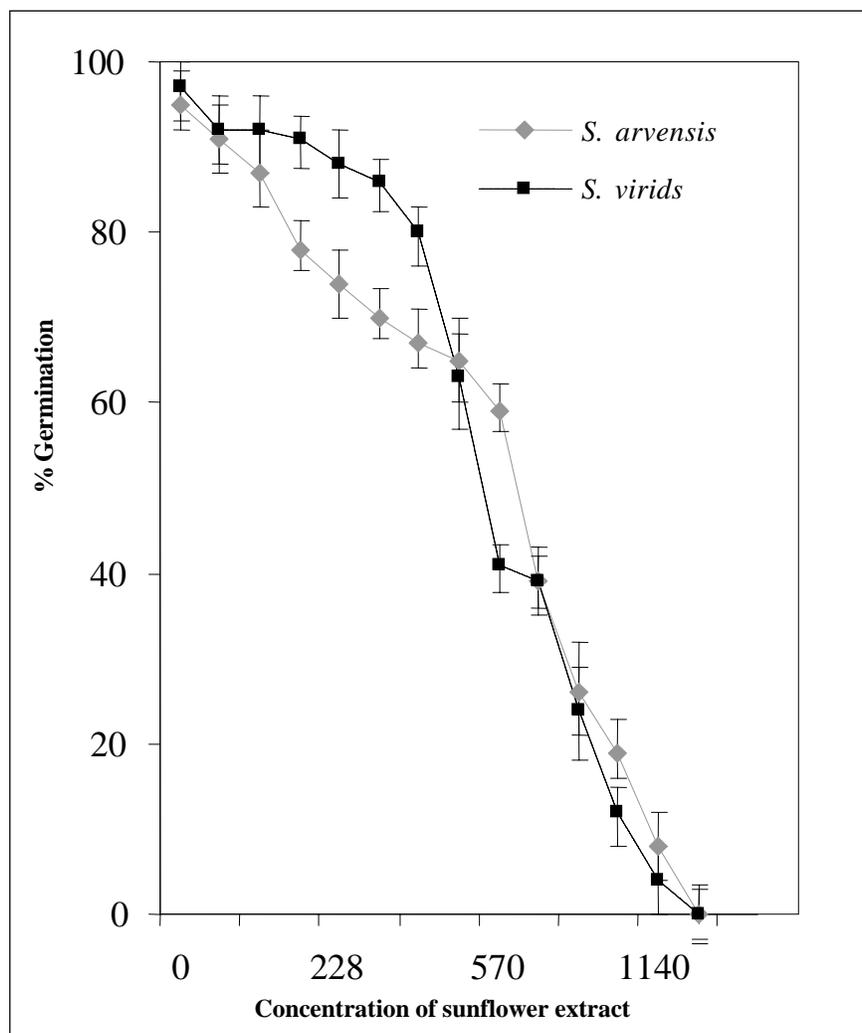


Fig. 1. Dose-response relationship curve showing the effect of sunflower extract on the germination of *Sinapis arvensis* and *Setaria viridis*. Data are mean of three replicates \pm S. D.

Table 1. Effect of sunflower extract on root and shoot length and dry weight of *Sinapis arvensis* and *Setaria viridis*

Concentration ($\mu\text{g/l}$)	Root length (cm)		Shoot length (cm)		Dry weight (mg/plant)	
	<i>S. arvensis</i>	<i>S. viridis</i>	<i>S. arvensis</i>	<i>S. viridis</i>	<i>S. arvensis</i>	<i>S. viridis</i>
0 (Control)	6.3a	4.2a	5.0a	3.65a	14.2a	11.3a
190	3.9b	1.01b	3.4b	1.8b	11.6b	7.8b
380	2.3c	0.66c	1.8c	0.88d	6.33c	3.2c
570	1.8d	0.42d	0.77d	0.71d	4.01d	2.9c
760	0.74e	0.27e	0.29e	0.18c	2.65e	1.7d

Data are mean of three replicates. Different letters in a column indicate difference at $P < 0.05$.

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). In this experiment also, root length was more affected compared with shoot length. At 190 $\mu\text{g/l}$ of sunflower added to soil, root length was reduced by nearly 42 and 34% in *S. arvensis* and *S. viridis*, respectively, whereas at 760 $\mu\text{g/l}$, it was reduced by nearly 75% in both the weedy species (Table 2). Here also, more inhibitory effects were observed on *S. arvensis* compared with *S.*

viridis. 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 *S. viridis* (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 *S. arvensis* compared with *S. viridis* (Table 3).

Table 2. Root and shoot length and dry weight of *Sinapis arvensis* and *Setaria viridis* grown in soil treated with sunflower extract

Concentration ($\mu\text{g/l}$)	Root length (cm)		Shoot length (cm)		Dry weight (mg/plant)	
	<i>S. arvensis</i>	<i>S. viridis</i>	<i>S. arvensis</i>	<i>S. viridis</i>	<i>S. arvensis</i>	<i>S. viridis</i>
0 (Control)	9.3a	6.4a	6.2a	4.65a	13.6a	12.3a
190	5.9b	3.4b	4.1b	2.2b	10.1b	8.6b
380	4.1c	1.7c	2.8c	1.4d	7.3c	4.2c
570	3.8c	0.82d	1.6d	0.95d	4.33d	2.7c
760	1.7d	0.3e	0.63e	0.38c	2.45e	1.5d

Data are mean of three replicates. Different letters in a column indicate difference at $P < 0.05$.

It is clear from the present study that sunflower exhibits an inhibitory effect on the germination and growth of both the test weed species—*S. viridis* and *S. arvensis*. From the dose-response studies curves, LC_{50} 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 (Dayan *et al.*, 2000). 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 (Picman, 1986; Pandey, 1996; Galindo *et al.*, 1999; Batish *et al.*, 2002). From the study, it is also clear that sunflower exerted more effect on root than on shoot. 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 were also reduced in the seedlings growing in treated soil

Table 3. Total chlorophyll and water content in 2-week-old seedlings of *Sinapis arvensis* and *Setaria viridis* grown in soil amended with sunflower

Treatment ($\mu\text{g/l}$)	Total chlorophyll (mg/g)		Water content (%)	
	<i>S. arvensis</i>	<i>S. viridis</i>	<i>S. arvensis</i>	<i>S. viridis</i>
0 (Control)	5.05a	3.4a	46.2a	44.65a
190	4.7b	2.1b	40.1b	39.2ab
380	4.1c	1.2c	32.8bc	34.4d
570	4.28c	0.82d	24.6c	28.6c
760	2.7d	0.3e	17.63d	23.1d

Data are mean of three replicates. Different letters in a column indicate difference at $P < 0.05$.

compared with untreated control. The reduction in chlorophyll content in response to allelochemicals has been reported in a number of plants (Einhellig and Rasmussen, 1979; Daizy and Kohli, 1991; Dayan *et al.*, 1999; Romagni *et al.*, 2000). 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 (1996) who reported that sunflower caused water loss due to root dysfunction. Thus, sunflower causes considerable toxicity to test weeds in soil too. However, much need remains to be done in this direction as regards its fate and dynamics in soil.

From the present study, it can be concluded that sunflower possesses weed suppressing ability that can be utilized for future weed management strategies.

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