

Full Length Research Paper

# Phytotoxicity of sunflower (*Helianthus annuus* L.) and its allelopathic potentiality on growth and yield attributes of *Parthenium hysterophorus*

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Recent developments in the weed science and allied aspects have involved several interdisciplinary approaches. In this context, indiscriminate use of herbicides for weed control has become a questionable subject, that besides controlling the weeds, the chemical herbicides are harmful in many ways to the soil, crops, other plants and the environment as a whole. To this end, pot and field experiments were conducted to test whether sunflower rhizosphere soil (SRSS) in pot and sunflower green manuring (SGM) in field experiments at various stages; 75, 90, 105 and 120 days after sowing (DAS) can reduce *Parthenium hysterophorus*. Sunflower cv. Myhco was screened at various stages viz. 75, 90, 105 and 120 days after sowing (DAS) through green manuring for field experiments and rhizosphere soil for pot experiments. Two cover crops, green gram (*Vigna radiata*) and pearl millet (*Pennisetum glaucum*) were used. In pot experiment, SRSS at various stages was compared with non sunflower rhizosphere soil (NSRSS). Growth and yield attributes of *P. hysterophorus* was compared between SGM and SGM along with cover crops and control in field experiments. Field experiments revealed that all growth and yield attributes of *P. hysterophorus* were inhibited maximum at 75 DAS of SGM with treatment SGM + pearl millet (PM) and the inhibitory effect decreased with increase of sunflower stage growing in the field. Pot experiments showed that SRSS at 75 DAS proved maximum inhibition to growth and yield attributes of *P. hysterophorus* as compared to higher stages. Allelochemicals were identified from sunflower through paper chromatography and subjected to spectroscopic analysis viz. ultraviolet, infra-red, nuclear magnetic resonance and mass spectra etc., for confirmation. These results suggest that sunflower green manuring and its rhizosphere soil has herbicidal potential and may be utilized as biological control of weeds for sustainable agriculture and environmental safeguard.

**Key words:** Green manuring, rhizosphere soil, allelochemicals, cover crops.

## INTRODUCTION

*Parthenium hysterophorus* (L.) Beauv. (bitter weed, fever few, congress grass, white top) is very common along the roadsides, around the agricultural fields and on wastelands. The allelopathic nature of this weed has been well documented and water soluble phenolics and sesquiterpene lactones have been reported from the roots, stems, leaves, inflorescences, pollens and seeds (Evans, 1997). The phenomenon of allelopathy where

one plant exerts a detrimental effect on another through the production of germination and growth inhibiting substances has been widely reported (Shaukat et al., 1983; Rizvi et al., 2000). It can play an important role in regulating plant diversity (Chou and Lee, 1991). Due to increase in the number of herbicide-resistant weeds and environmental concerns in the use of synthetic herbicides, there have been considerable efforts in designing alternative weed management strategies. The conventional synthetic herbicides are becoming less effective against the resistant weed biotypes. Herbicides have been shown to control weeds effectively, but the

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intensive and extensive use of herbicides have resulted in the development of resistant population (Gealy et al., 2003; Beckie, 2006) and contamination of agricultural produce and environment (Buhler et al., 2000; Clausen et al., 2007). Thus, interest in alternative methods to control *P. hysterophorus* increased recently (Dharmraj and Sheriff, 1994a; Dharmraj, 1994c; Rawat, 2002; Singh, 2002; Anjun et al., 2005; Azania et al., 2003).

Allelopathic potential of sunflower for weed control has been reported in its cultivars viz. Ramsum HS-52, Peredovik, Hybrids 201, 8941. In green house studies, sunflower 'Russian mammoth' reduced both seed germination and biomass of weeds (Hall et al., 1982). The aqueous extracts as well as growing plant inhibit the seed germination and seedling growth of *Abutilon theophrasti*, *Datura stramonium*, *Ipomoea* species and *Brassica kaber* (Dharmraj et al., 1994a, b, c; Leather and Forrence, 1979; Prusty et al., 1994; Wilson and Rice, 1968). Its aqueous extracts reduced the germination (36 to 56%) and seedling growth (22 to 57%) of *Trianthema portulacastrum*, *Amaranthus viridis* and *P. hysterophorus*. However, in field studies, drastic reduction occurred in germination (83 to 95%), growth (79 to 95%) and chlorophyll content of the above weeds and also in *Portulaca oleracea* and *Flaveria australasica* weeds (Dharmraj et al., 1994a, b, c). Sunflower-oat rotation over five years period significantly lowered the density of grassy and broadleaf weeds in fields than in control plots (Leather, 1983a). Although weed density increased in all plots over the five seasons, the rate of increase was less in sunflower plots. There was, however, little difference among the various sunflower cultivars. In further studies, weed biomass was equivalent in plots planted with sunflower, whether S-thydidipropyl carbomothioate (EPTC) herbicide was applied or not, clearly showing the efficacy of sunflower mediated weed control (Leather, 1987). In sunflower-wheat rotation field trials, sunflower decreased the density and dry weight of wild oat and *Cirsium arvense* in the following wheat crop (Cernusko and Borkey, 1992). Soil incorporation of sunflower residues significantly reduced the number of dicot weeds by 66% compared with the control (Anaya, 1989). In pot experiments, sunflower straw depressed the plant height of wild oat, *Agropyron repens*, barnyard grass, *Ambrosia artemisiifolia* and lambsquarter and decreased the biomass of the last three weed species (Muminovic, 1991). Application of sunflower residue at 2 tons per hectare or its preceding crop reduced pollution of *Cleome viscosa*, *Corchorus trilocularis* and *Cyperus iria*.

The potential of sunflower as source of allelochemicals is well known (Varela, 1982). Bioassays of leaf aqueous extracts show strong inhibition and stimulation in germination and root length of test plant species. The leaf aqueous extracts of sunflower 'SH 222' were found to obtain five new guaianolides and the annuolides possess allelopathic activity of sunflower. All the guaianolides possess allelopathic activity over dicotyledonous species

and are likely to be involved in the allelopathic activity of sunflower cultivars (Macias et al., 1993). Macias et al. (1996) characterized absolutely new allelochemicals viz. 16 sesquiterpene lactones, five flavonoids, four kaurene diterpenes, 14 bisnorsesquiterpenes and sesquiterpene heliannulos. The heliannulos proved inhibitory to dicot weed species and hence may be an excellent source as a pre and post emergence herbicides at very low doses ( $10^{-4}$  to  $10^{-9}$ ).

The objective of this study was to investigate the hypothesis that utilization of sunflower as natural herbicide may reduce *P. hysterophorus* infestation. The specific objectives were (a) to develop an environmental friendly weed management practices so as to reduce the yield losses in field crops caused by the weeds and (b) to overcome the problems associated with the present herbicides.

## MATERIALS AND METHODS

Sunflower variety cv. Mahyco was used for green manuring and its rhizosphere soil was used for field and pot experiments respectively and crops namely *V. radiata*, W.K.-851 (green gram), and *Pennisetum glaucum* Cr. HHB – 67 (pearl millet) were used as cover crop for only field study. Seeds of all crops were procured from Haryana Agricultural University, Hisar, Haryana. Seeds of *P. hysterophorus* were collected from its natural population in adjoining areas of experimental farm.

### Pot experiment

This experiment consisted to two components; i) plant component: that is test crops plant species (*V. radiata*, *P. glaucum*) and test weeds *P. hysterophorus* and ii) soil components: two types of soil; the non-sunflower rhizosphere soil (NSRSS) soil from the field where sunflower was not grown (control) and sunflower rhizosphere soil (SRSS). The treatments were replicated thrice in completely randomized design. The soil used in pot trial was sandy loam in texture, alkaline pH (8.4) and poor in C (0.25%). Soil for control treatment was brought from the field where sunflower was not growing. For treatment pots, soil was gotten from sunflower plants rhizosphere from a depth of 30 cm at different stages viz. 75, 90, 105 and 120 days after sowing (DAS) to determine the phytotoxicity of sunflower rhizosphere soil growing in the field on *P. hysterophorus*.

Immediately after harvesting sunflower plants, the soil was dug and clods were crushed to powder. All the soil was sieved through a 2 mm-mesh sieve to remove all the plant residues. All the pots (25 cm diameter) were lined with polythene sheets to prevent adsorption of allelochemicals by pots or through leakage of leachates from the pots. The pots were filled with soil at 4.0 kg/pot on dry weight basis as per the treatments. Recommended doses of nitrogen and phosphorous was also applied. The pot soil was leveled and pressed with hand to remove air pockets and was irrigated with 900 ml tap water. The next day, as per treatments, five seeds of test crop were sown (kept on soil surface) per pot. For test weeds, the soil infested with *P. hysterophorus* seeds was used respectively. Immediately, seeds were covered with 500 g dry sieved soil per pot to prevent crust information over the germinating seeds. After the completion of germination, the pots were irrigated with tap water as per requirement. To determine the phytotoxicity on particular crops and weeds, the unwanted weeds growing in the

pots were eliminated. The thinning of plants in pots was done at 15 DAS and five crop plants were kept per pot.

### Field experiment

Sunflower plant cv. Mahyco was grown in the experimental field of Agronomy farm, HAU, Hisar, to determine the effect of green manuring of sunflower whole plant incorporation on weed *P. hysterophorus*. The green manuring of sunflower followed by cover crops; *V. radiata*, W.K.-851; *P. glaucum* Cr. HHB – 67 were used for the experiment. Sunflower plants were harvested at various stages of its growth in the field; 60, 75, 90, 105 and 120 days after sowing (DAS). The weighed amount of harvested sunflower plant at various stages was chopped and incorporated into the soil of treatment plots SGM+PM and SGM+GG but not in the plots of the control. The plots were irrigated for a short period and the chopped tissues of sunflower were mechanically mixed with the soil to 15 cm soil depth in the month of April, 2000 and 2002. Prior to green manuring in treatment plots, the soil was loamy-sandy with sand 76%, silt 15.8%, clay 18.6%, N 66.1 kg/ha, P 36.8 kg/ha and k 344 kg/ha and soil pH 8.12.

The field experiment was a completely random block design with ten treatments and with four replicates. Plots were 2.5 m by 2 m and were separated by 30 cm buffer strips. For the cover crops, *V. radiata*, W.K.-851; *P. glaucum* Cr. HHB – 67 were sown at distance of 25 cm between rows and 20 cm between hills within the same rows. For *P. hysterophorus* seeds, soil from natural seed bank where the selected weed was growing naturally near to the experimental site was collected and sieved through 2 mm mesh to avoid unwanted materials in the experimental plots. Sieved soil of *P. hysterophorus* seeds was incorporated in the treatment plots. Three seeds of *V. radiata*, W.K.-851 and *P. glaucum* Cr. HHB – 67 as per experimental design were sown in each hill. No chemical herbicides were applied in the experimental plots, but other weeds that occurred in the plots except *P. hysterophorus* were removed by hand during the experiment. A basal dose of nitrogen and phosphorus (60 kg/ha) was drilled at sowing when the field was ploughed. After completion of germination, the plots were irrigated. At maturity, plant population, height, grain yield, plant population, tillering/branches/ number of capitulum/plant, biomass yield/ plant and per plot were measured using 1 m<sup>2</sup> quadrat at three samples point per plot.

### Estimation of total phenolics

The phenolic compound in plant materials of sunflower was determined using the method of King and Health (1967) and Allen et al. (1974). Total soluble polyphenolics were analyzed by Folin-Ciocalteu reagent according to Swain and Hillis (1959). The polyphenolics include both hydrolysable tannins and non-tannins polyphenolics. 0.5 g sample of different materials was extracted with 50 ml of 50% methanol by heating the samples on a water bath at 77 to 80°C for 1 h. The extract was used for determining the concentration of polyphenols by using Folin-Denis reagent and 12% Na<sub>2</sub>CO<sub>3</sub> and reading the absorbance at 760 nm.

### Identification of potential allelochemicals in leaf debris using high-performance liquid chromatography (HPLC)

Phenolic constituents in the plant material of sunflower were extracted using the method of Harborne (1973). Plant material (10 g) of sunflower was hydrolyzed in 50 ml of concentrated 2 N HCl for 30 min, cooled and extracted with 10 ml of ethyl acetate two times.

The ethyl acetate extracts were filtered through Whatman number 44 filter circle, pooled and concentrated. The left over residue was extracted in 5 ml of HPLC grade methanol, filtered through 0.45 µm nylon filter and analysed by (HPLC). HPLC was performed on a waters liquid chromatograph 2695 (Waters Associates, Millford, MA) fitted with a binary gradient pump using a reverse phase C8 column (4.6 × 150 mm) and injection loop SM7. The solvent system used consisted of 0.01 M hexane sulfonic acid sodium salt buffer at pH 4.0 (solvent A), and acetonitrile (solvent B) at a ratio of 60:40 (v/v). The flow rate was 1 ml min<sup>-1</sup> and UV detector (model 2487; Waters Associates, Millford, MA) was set at a wavelength of 230 nm. The other HPLC operating conditions included sample volume of 20 µl, chart speed of 0.25 cm min<sup>-1</sup>, detector sensitivity of 1.0 AUFS, recorder range of 10 mV FS and the ambient column temperature. The different phenolic acids were identified and their relative concentrations were calculated by comparing the peak areas of the samples with those of the standards procured from Sigma, St. Louis and Lancaster, UK.

### Statistical analysis

The average of four samples from each replication for germination, plant population, grain yield/plant/plot and biomass yield/plant/plot was used for statistical analysis. The percent germination data was subjected to angular transformation for statistical analysis. One way ANOVA was performed on each dependent variable test crops in field and pot experiment by using general linear model (GLM) design in the SPSS V. 10.0. Critical difference (CD) at 5% confidence level was used for comparison of treatment.

## RESULTS

### Pot study

#### Plant height

In general, the sunflower rhizosphere soil at various stages significantly decreased the height of *P. hysterophorus* as compared to non-sunflower soil (control) (Table 1). At 60 DAS of sunflower, the rhizosphere soil was found most inhibitory to *P. hysterophorus* (65.5%). The magnitude of inhibition to height in *P. hysterophorus* at all stages followed the order 60 (65.8%) > 75 (55.5%) > 90 (41.4%) > 105 > (38.8%) > 120 (18.7%) days after sowing, respectively (Table 1).

#### Plant population

At 60 DAS of sunflower, the rhizosphere soil showed maximum decrease in the number of plants of *P. hysterophorus* as compared to higher stages studied (Table 1). The plant population of *P. hysterophorus* was observed most sensitive to rhizosphere soil and inhibited maximum at 60 DAS (61.5%), whereas at 105 DAS no inhibitory effect was observed. The magnitude of inhibition to plant population of *P. hysterophorus* at all stages followed the order; 60 (61.5%) > 75 (48.5%) > 90 (28.5%) > 105 > (0.0%) > 120 (17.6%) days after sowing, respectively (Table 1).

**Table 1.** Effect of phytotoxicity of sunflower rhizosphere soil at various stages of crop growth on *Parthenium hysterophorus*.

Treatment	Plant height	Plant population	Number of branch	100-seed weight (g)	Yield /plant (g)		Yield /pot (g)	
					Grain	Straw	Grain	Straw
<b>Sunflower rhizosphere soil 60 DAS</b>								
NSRSS	82.3	7.8	8.8	0.25	1.12	7.18	2.18	52.3
SRSS	28.1 (-65.8)	3.0 (61.5)	4.5 (-48.8)	0.23 (-8.0)	0.62 (-44.6)	3.30 (-54.0)	0.96 (-59.9)	22.3 (-57.3)
CD at 5%	2.45	2.21	1.35	NS	0.04	0.89	0.68	0.19
<b>Sunflower rhizosphere soil 75 DAS</b>								
NSRSS	81	6.8	9.15	0.27	1.15	7.15	2.58	49.0
SRSS	36 (-55.5)	3.5 (-48.5)	5.0 (-45.0)	0.23 (-14.8)	0.75 (-34.7)	4.0 (-43.6)	1.15 (-55.4)	29.15 (-40.6)
CD at 5%	1.90	1.27	2.51	NS	0.45	1.34	0.71	0.19
<b>Sunflower rhizosphere soil 90 DAS</b>								
NSRSS	82	7.0	8.50	0.26	1.16	7.0	2.60	48.3
SRSS	48 (-41.4)	5.0 (-28.5)	6.5 (23.5)	0.24 (-7.6)	0.95 (-18.1)	5.15 (-27.1)	1.15 (-48.4)	31.25 (-35.4)
CD at 5%	0.84	0.74	0.96	NS	0.11	0.62	0.71	0.11
<b>Sunflower rhizosphere soil 105 DAS</b>								
NSRSS	80	6.5	8.0	0.24	1.0	7.0	2.60	43.2
SRSS	45 (-38.8)	6.5 (0.0)	7.5 (-6.2)	0.24 (0.0)	1.0 (0.0)	5.56 (-21.4)	1.35 (-56.0)	13.0 (-23.4)
CD at 5%	0.61	0.74	NS	NS	NS	0.26	0.64	0.67
<b>Sunflower rhizosphere soil 120 DAS</b>								
NSRSS	81.3	8.5	9.0	0.25	1.19	7.85	2.29	45.3
SRSS	66.0 (-18.7)	7.0 (-17.6)	8.0 (-11.1)	0.24 (-4.0)	1.10 (-17.5)	6.10 (-21.7)	2.0 (-9.0)	39.0 (-13.7)
CD at 5%	0.9	0.11	0.09	NS	0.08	0.24	0.06	0.08

CD at 5% = Critical difference at 5%; DAS = days after sowing; NS= non-significant. Data in parenthesis indicate % stimulation (+) and inhibition (-) over control.

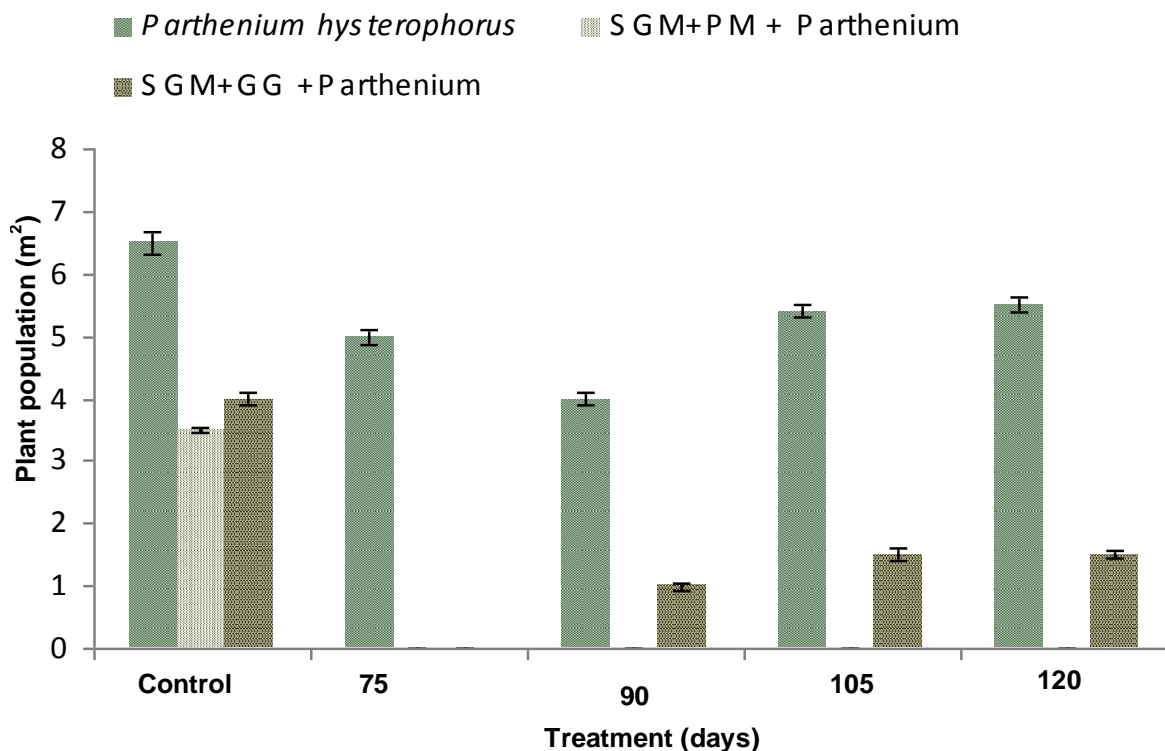
### Number of branches/tillering

The results reveal that the effect of rhizosphere soil to tiller in *P. hysterophorus* has greater effect throughout all stages (60, 75, 90, 105 and 120 DAS). The magnitude of inhibition to tillering in *P.*

*hysterophorus* from rhizosphere soil of sunflower at all stages followed the order: 60 (48.8%) > 75 (48.5%) > 90 (28.5%) > 120 (11.1%) > 105 (6.2%). The results indicate that the stage 120 DAS was more harmful to tillering in *P. hysterophorus* as compared to 105 (Table 1).

### Seed weight

75 DAS of sunflower rhizosphere soil proved to be most inhibitory to seed weight of *P. hysterophorus* (14.8%), whereas 105 DAS was observed to be less inhibitory (0.0%). The magnitude of inhibition



**Figure 1.** Plant population of *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).

to seed weight in *P. hysterophorus* followed an order 75 (14.8%) > 60 (8.05%) > 90 (7.6%) > 120 (4.0%) > 105 (0.0%), respectively (Table 1).

### Grain yield

Except 105 DAS, all stages of sunflower rhizosphere soil inhibited grain yield of *P. hysterophorus* per plant. Maximum phytotoxicity of rhizosphere soil grain yield was observed at 60 DAS (44.6%) (Table 1). The trend of inhibition of rhizosphere soil to grain yield followed an order 60 (44.6%) > 75 (34.7%) > 90 (18.8%) > 120 (17.5%) > 105 (0.0%), respectively (Table 1) whereas in per pot, maximum phytotoxicity of rhizosphere soil to grain yield was observed at 60 DAS (59.9%) (Table 1). The trend of inhibition of rhizosphere soil to grain yield followed an order 60 (59.9%) > 75 (55.4%) > 105 (56.0%) > 90 (48.4%) > 120 (9.0%), respectively (Table 1).

### Biomass yield

Maximum inhibition in biomass yield of *P. hysterophorus* was observed at 60 DAS (54.6%) (Table 1). The trend of inhibition of rhizosphere soil to biomass yield followed an order 60 (44.6%) > 75 (34.7%) > 90 (18.8%) > 120

(17.5%) > 105 (0.0%), respectively (Table 1) whereas, in per pot, maximum inhibition in biomass yield of *P. hysterophorus* was observed at 60 DAS (57.3%) (Table 1). The trend of inhibition of rhizosphere soil to biomass yield followed an order 60 (57.3%) > 75 (40.6%) > 90 (35.4%) > 105 (23.4%) > 120 (13.7%), respectively (Table 1).

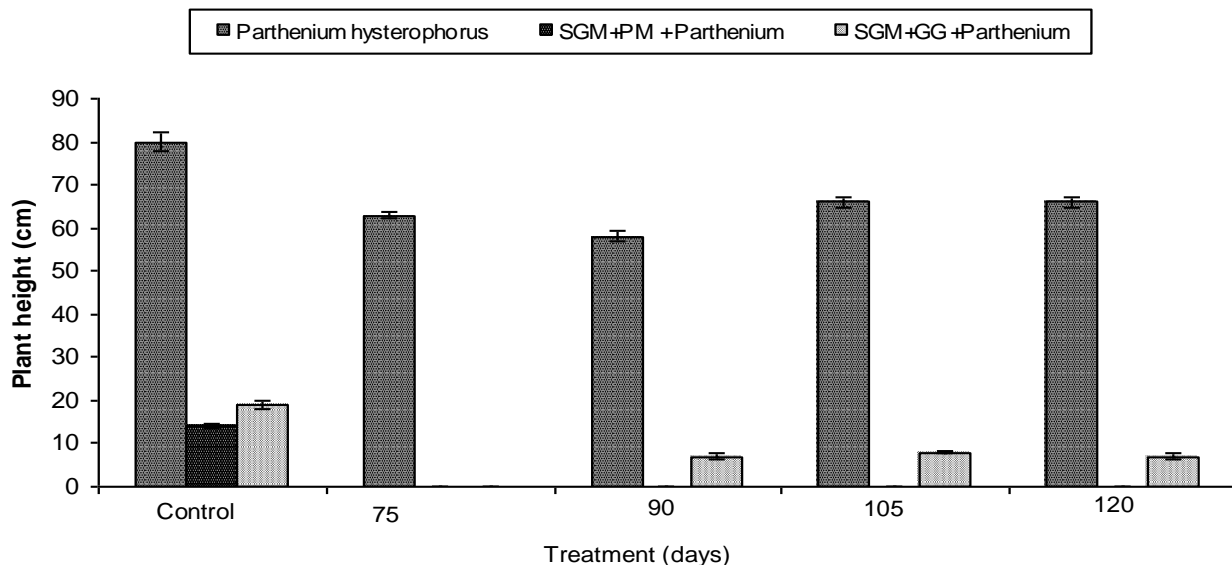
### Field experiment

#### Plant population

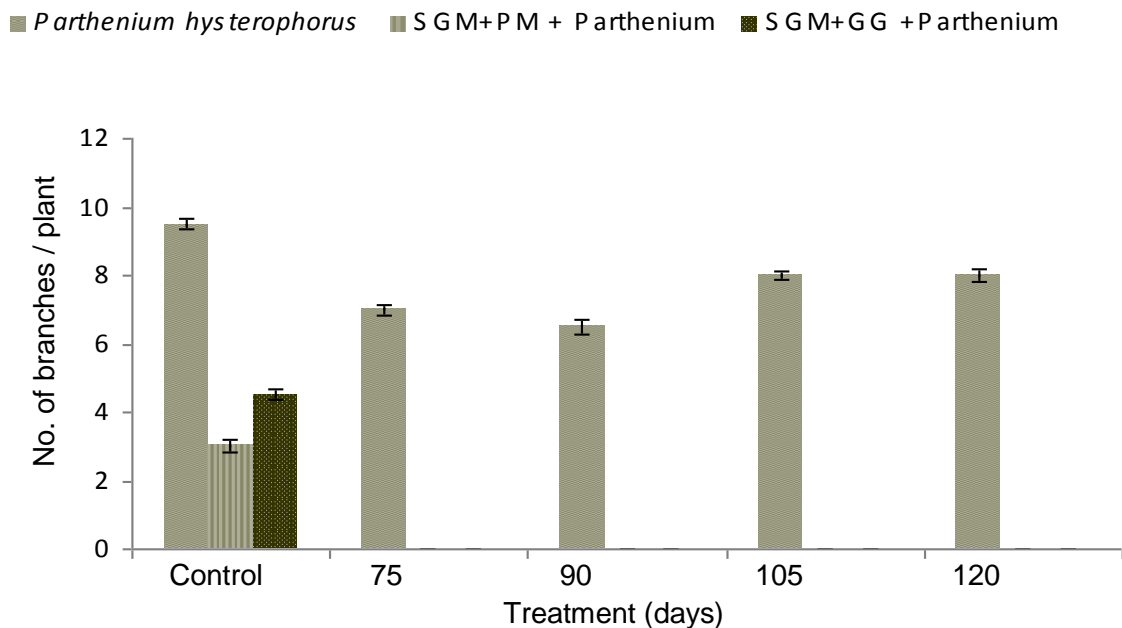
With plots GM+PM, population of *P. hysterophorus* was completely inhibited (100%) (Figure 1,  $P < 0.05$ ) in all stages of sunflower green manuring as compared to control plots. Plant population of *P. hysterophorus* with GM+GG also significantly reduced in all stages of sunflower green manuring as compared to the control. Green manuring of sunflower at all stages without cover crops significantly reduced population of *P. hysterophorus* as compared to the control (Figure 1,  $P < 0.05$ ).

#### Plant height

Under GM+PM, height of *P. hysterophorus* was



**Figure 2.** Plant height of *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).

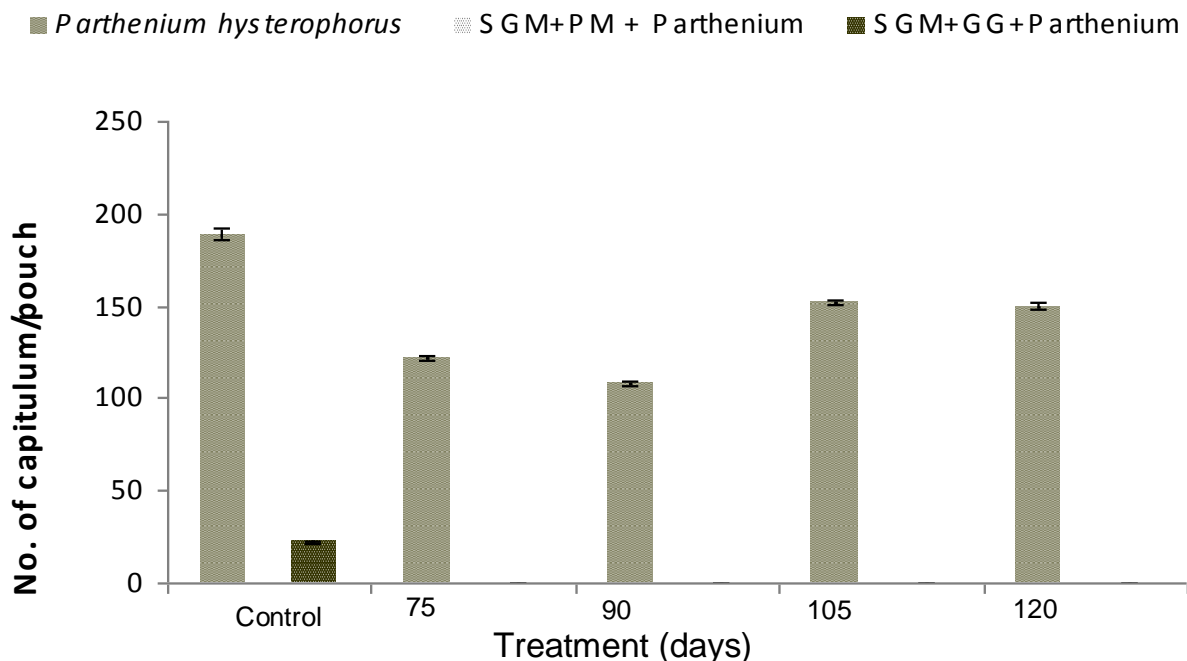


**Figure 3.** Number of branches in *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG: green gram (*Vigna radiata*).

completely inhibited (100%) (Figure 2,  $P < 0.05$ ) in all stages of sunflower green manuring as compared to the control plots. Plant height of *P. hysterophorus* with GM+GG also significantly reduced in all stages of sunflower green manuring as compared to the control. Green manuring of sunflower at all stages without cover crops significantly reduced height of *P. hysterophorus* as compared to the control (Figure 2,  $P < 0.05$ ).

**Number of branches/tillering**

Branches of *P. hysterophorus* was found completely absent (100% inhibition) under GM+PM (Figure 3,  $P < 0.05$ ) in all stages of sunflower green manuring as compared to the control plots. Branches in *P. hysterophorus* with GM+GG also significantly reduced in all stages of sunflower green manuring as compared to



**Figure 4.** Number of capitulum/pouch in *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).

the control. Green manuring of sunflower at all stages without cover crops significantly reduced branches of *P. hysterophorus* as compared to the control (Figure 3,  $P < 0.05$ ).

#### Number of capitulum

Compared with the control, capitulum of *P. hysterophorus* was found to be 100% reduced under GM+PM and GM+GG (Figure 4,  $P < 0.05$ ) at all stages of sunflower green manuring. Under GM without cover crop, maximum reduction of pouch of *P. hysterophorus* was observed at 90 DAS (42.8%) followed by 75 (35.4%) > 120 (20.6%) > 105 (-19.5%), respectively (Figure 4,  $P < 0.05$ ).

#### Grain yield

100% reduction of grain yield of *P. hysterophorus* was found under SGM+PM at all stages of sunflower green manuring (Figures 5 and 7,  $P < 0.05$ ). Also, under SGM+GG, there was 100% significant reduction of grain yield of *P. hysterophorus* at all stages except 105 DAS of sunflower green manuring.

#### Biomass yield

100% reduction of biomass yield of *P. hysterophorus* was

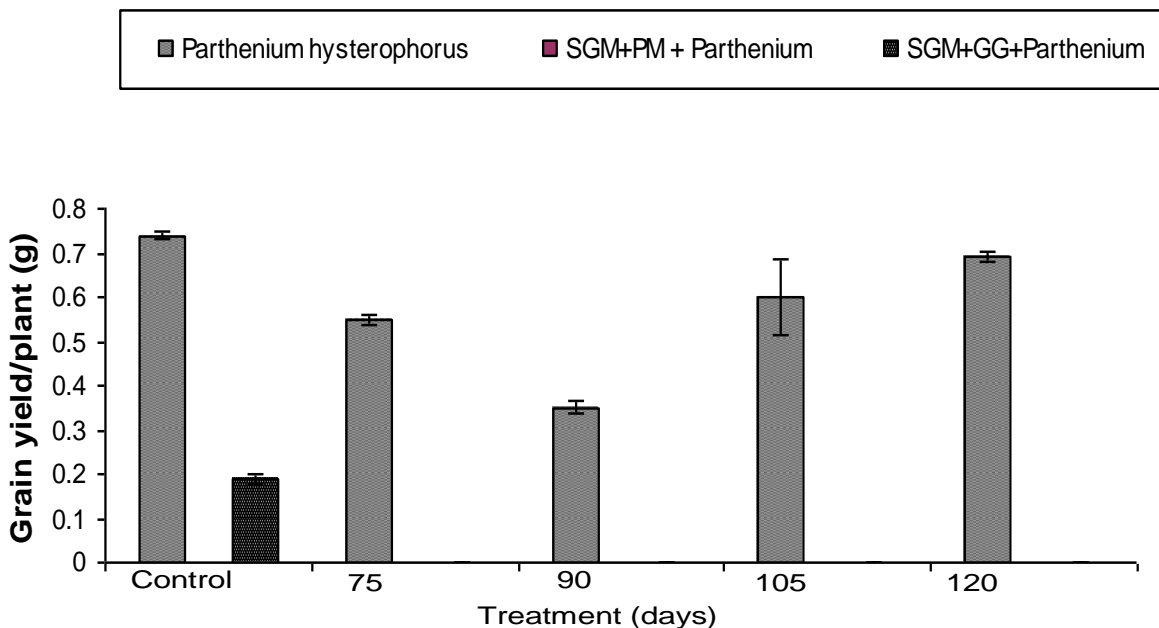
found under GM+PM at all stages of sunflower green manuring (Figures 6 and 8,  $P < 0.05$ ). Under GM+VR also, 100% significant reduction in biomass yield of *P. hysterophorus* at the stage of 75 and 105 DAS was observed, whereas biomass at 90 and 120 DAS resulted in 85.2 and 22.3% reduction, respectively as compared to the control (Figure 8,  $P < 0.05$ ).

#### Quantification of phenolics in rhizosphere soil and plant extract of sunflower

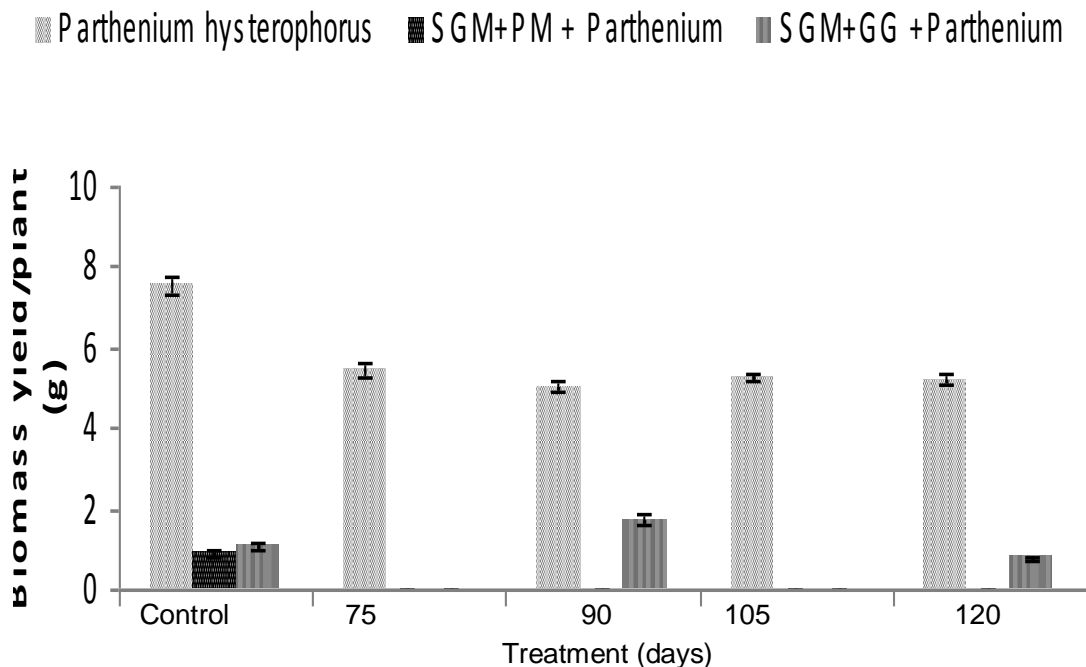
Phenolics are the most common water-soluble allelochemicals and play a significant role in plant-plant interactions including allelopathy. The results show that the amount of phenolics was more ( $849.49 \pm 19.25$ ) at 2% concentration of aqueous extract in sunflower plant residue as compared to the rhizosphere soil ( $132.32 \pm 5.65$ ) (Table 2).

#### Identification of phenolic acids in plant residue of sunflower

Upon HPLC analysis, a total of nine phenolic compounds viz. ferulic acid, p-coumaric acid, syringic acid, chlorogenic acid, isochlorogenic acid, neochlorogenic acid, vanillic acid, p-hydroxybenzoic acid and caffeoylquinic acid were identified in the plant residue of the sunflower. The results reveal that the maximum composition percent



**Figure 5.** Grain (seed) yield in *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).



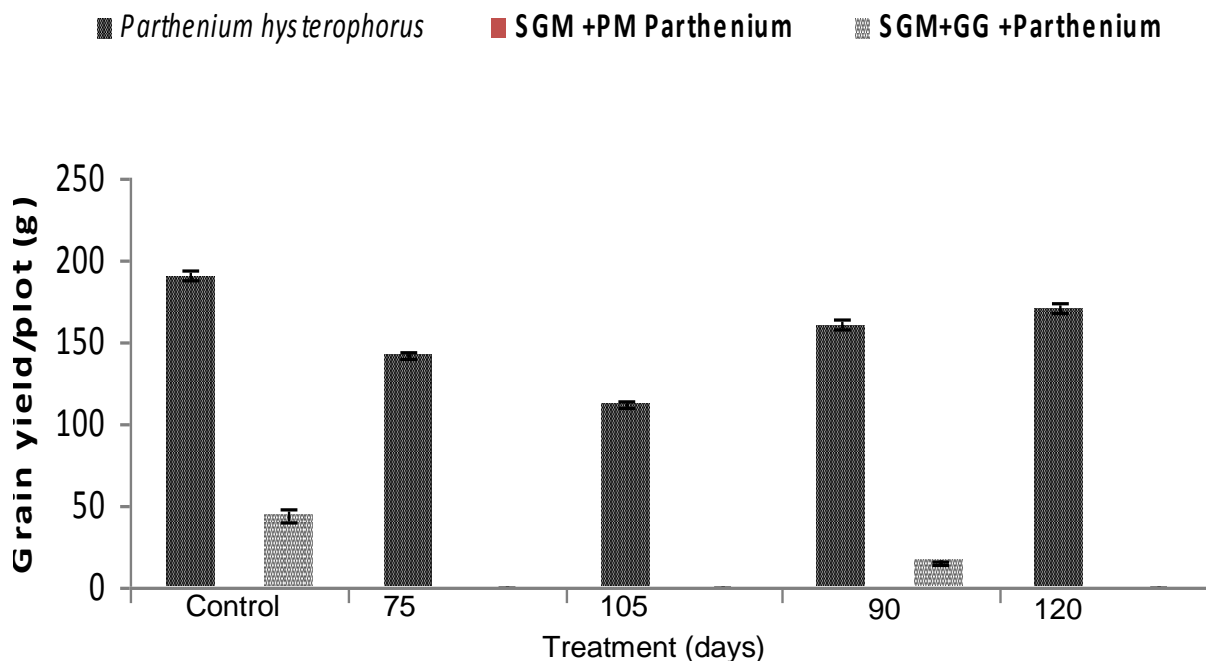
**Figure 6.** Biomass yield/plant in *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).

was recorded for syringic acid phenolic compound, whereas minimum composition percent among the identified phenolic compounds was found for p-hydroxybenzoic acid (Table 3.)

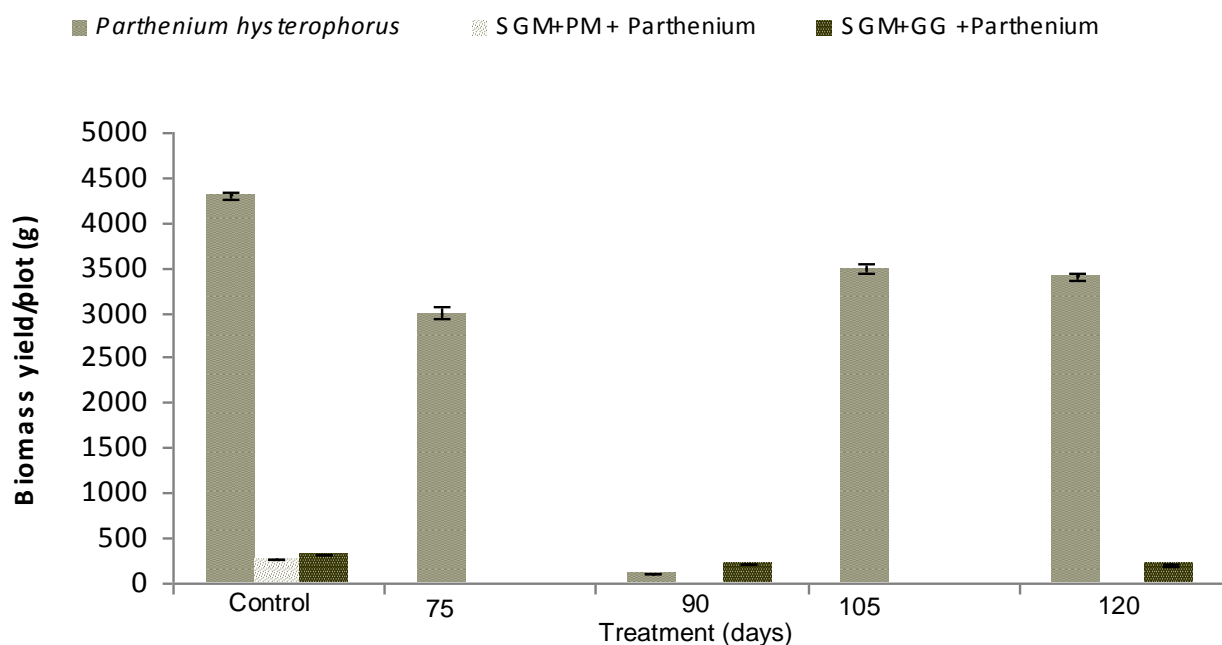
### DISCUSSION

Our experiments reveal that *P. hysterophorus* infestation in the field and adjoining areas could be reduced through





**Figure 7.** Grain (Seed) yield/ plot in *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).



**Figure 8.** Biomass yield/plot in *P. hysterophorus* under sunflower green manuring at different stages in field experiment. SGM, Sunflower green manuring; PM, pearl millet (*Pennisetum glaucum*); GG, green gram (*Vigna radiata*).

sunflower green manuring and its rhizosphere soil. In pot experiment, sunflower rhizosphere soil reduced all parameters viz. plant height, population and grain and biomass yield of *P. hysterophorus*. The applied soil

proved most inhibitory at initial 60 DAS and the effect of inhibition was observed upto 90 DAS of sunflower. After that, the rhizosphere soil proved less inhibitory to *P. hysterophorus*. Sunflower rhizosphere soil proved more

**Table 2.** Amount of phenolics in the rhizosphere soil of *Helianthus annuus* ( $\mu\text{g}^{-1}$ ) and plant extracts ( $\mu\text{mL}^{-1}$ ).

Treatment	Amount of phenolic
Control (where sunflower was not grown)	9.22 $\pm$ 1.02
Sunflower rhizosphere soil	44.32 $\pm$ 2.65*
<b>Plant extracts</b>	
0.5	265.13 $\pm$ 8.32 <sup>a</sup>
1.0	546.85 $\pm$ 14.56 <sup>b</sup>
2.0	849.49 $\pm$ 19.25 <sup>c</sup>

Different letters represent significant difference at  $P \geq 0.01$ . \*Means significantly different from control at  $P \geq 0.01$ .

**Table 3.** Identification and relative amounts ( $\pm$ ) of phenolic acids in plant residues of *Helianthus annuus*.

Phenolic compound	Retention time (min)	Composition (%)
Ferulic acid	1.40	32.21 $\pm$ 1.56
P-Coumaric acid	1.60	29.52 $\pm$ 1.09
Syringic acid	1.50	34.11 $\pm$ 1.27
Chlorogenic acid	1.80	28.47 $\pm$ 1.10
Isochlorogenic acid	1.98	31.26 $\pm$ 1.21
Neochlorogenic acid	2.53	30.29 $\pm$ 1.88
Vanillic acid	3.14	2.65 $\pm$ 0.23
P-Hydroxybenzoic acid	4.52	2.56 $\pm$ 0.14
Caffeoylquinic acid	3.65	11.36 $\pm$ 0.86

harmful at 60 DAS to plant height (65.8%) in pot study and the magnitude of this effect was observed to be lower as the stages of sunflower goes higher. The harmful effect of such soil might be due to the presence of allelochemicals of sunflower released by roots into the soil. Waller and Nowacki, (1978) reported the presence of P-hydroxy benzoic and benzoic acids in the sandy soil of a Florida citrus orchard in which citrus was badly affected with the die back diseases. The source of phytotoxic substances in the rhizosphere soil could be the exudates of root and/or microbial toxins. Pariana (1992) reported that the various organic extracts derived from sunflower leaves inhibited the germination and rooting of the stem cuttings of *Parthenium* weed, leading to the checking of its reproductive potential. The increased retardation leading to inhibition of morphogenic function at higher concentrations of *Helianthus annuus* is viewed or linked to greater release of phytotoxins from leaves on their decomposition. Sandhu (1997) also reported that phytotoxicity of soil incorporated residues of sunflower at various stages of plant growth.

Furthermore, the study was extended to field experiments where sunflower green manure under treatment SGM+PM proved to be most inhibitory to growth and yield parameters of *P. hysterophorus*, whereas treatment SGM+GG proved to be inhibitory to *P. hysterophorus* but less inhibitory as compared to SGM+PM treatment. The

magnitude of inhibition by green manuring was observed maximum at 75 to 90 DAS and decreased with increase of stages of sunflower plant growing in the field. This might be due to higher occurrence of the resistant materials like lignin, cellulose, hemicelluloses and polyphenols at older age as compared to younger one. Due to these resistant materials, the plant biomass could not be easily decomposed as much as in the young age. Gill and Sandhu (1993) studied sunflower, maize, cotton, pigeon pea, soybean and pearl millet. The seeds were sown in pots containing ground sunflower leaves from a mature crop incorporated into the soil (0.5 to 2.5% w/w basis). Decomposing sunflower leaves decreased the sunflower seed germination in all other crops. While the shoot and root growth responses to allelopathic effects were dependent on the species, the adverse growth effects on all the species were evident at the higher concentrations. Kulvinder et al. (1999) reported that mungbean (*V. radiata*) cv. K-851 and pearl millet (*Pennisetum typhoides* [*P. glaucum*] cv. HHB-67) germinated on sandy loam soil with 1.5 or 3.0 t ground sunflower residues (stems or leaves) with or without N. The residues initially did not affect germination. However, germination was reduced significantly in the first week, with greater reductions with higher residue rate and with N, the germination percentages returned to normal after 2 to 3 weeks. Pariana (1992) also reported that seeds of *P.*

*aureus* var. ML-267, *C. arietinum* var. C-235, *Trigonella foenumgraecum* var. HFM-65 and *P. americanum* var. HHB-60 germinated when sown in sunflower green manure free soil (control). In contrast, none of the seeds of any of these crops sown in 0.25, 0.5: 1.0 (w/w) manure-soil mixtures germinated upto 20 days.

Phenolics are the most common water-soluble allelochemicals and play a significant role in plant– plant interactions including allelopathy (Mizutani, 1999; Batish et al., 2006b, 2007a, b). In this study, an appropriate water soluble phenolic compound was found in the plant residue and rhizosphere soil of the sunflower. Upon HPLC analysis, nine phenolic compounds viz. ferulic, p-coumaric acid, syringic acid, chlorogenic acid, isochlorogenic acid, neochlorogenic acid, vanillic acid, p-hydroxy benzoic acid and caffeoylquinic acids were identified from plant residues of the sunflower. All these phenolic acids are known as phytotoxins and widely implicated in allelopathic studies, and even effected the growth of weeds (Dharmraj, 1998; Leather, 1983a, 1987). Macias et al. (1996) reported that in addition to known phenolic compound in sunflower, they characterized new 16 sesquiterpene lactones (nine glucanolides and seven germacranolides), five flavonoids, four kaurenoids diterpenes, four bisnorsesquiterpenes and two novel families of sesquiterpene Heliannuoles (15 compounds) and Heliespinores (four compounds). Park et al. (1992) reported that hydroquinone, beta-resorcylic acid, vanillic acid, caffeic acid, salicylic acid and quercetin were characterized from the acidic fraction of root exudates from sunflower. Hydroquinone, gentisic acid, beta-resorcylic acid, vanillic acid, caffeic acid, ferulic acid and quercetin were elucidated from the neutral fraction. Sandhu (1997) reported that the chromatographic, physicochemical and spectral analysis showed the presence of chlorogenic and isochlorogenic acids in sunflower. Pariana (1992) reported that the allelochemicals from sunflower are polar and non-polar in nature. Two terpenoid compounds (A and B) were isolated and characterized from the relatively more allelopathic fractions. Both these compounds (A and B) showed allelopathic activity and inhibited germination parameters viz., photosynthesis and respiration of the target plant, *Phaseolus aureus* var. ML-267.

Furthermore, chemical studies on different parts of *H. annuus* have led to the identification of a number of compounds; prominent among them being the sesquiterpene lactones. Spring et al. (1982) isolated two sesquiterpene lactones names niveusin C and 15-hydroxy-3-dehydroxy fruticin from leaves and stems of *H. annuus*. Both sesquiterpene lactones strongly inhibit indole-3-acetic acid (IAA) induced elongation growth of *Avena sativa* L. coleoptile segments and *H. annuus* L. hypocotyls segments. Further investigations on growth inhibiting substances from young leaves and the apical part of stem of *H. annuus* resulted in the extraction of three additional sesquiterpene lactones (Spring et al., 1982). Spring et al. (1989) later identified six sesqui-

terpene lactones by HPLC separations from capitate glandular trichomes of *H. annuus* L. There has been a general understanding among the warranty weed that extensive efforts are required to reach out the active allelopathic compounds and the actual ratio of the compounds in the plant extracts released by crops, so that the magnitude of the combined effect of allelochemical mixtures in weed suppression could be elucidated. Herein, sunflower green manure in field experiments and rhizosphere soil in pot experiments were observed to be harmful to *P. hysterothorus* in field and pot experiments. We can infer from this study that sunflower biomass, as well as rhizosphere soil could be utilized for weed management.

## Conclusion

Our results show that sunflower green manuring and rhizosphere soil has potential to reduce plant height, plant population, number of capitulum and biomass of *P. hysterothorus* compared with the control. Sunflower green manuring at various stages with cover crop PM was observed to be most inhibitory. The effect of sunflower green manuring was more inhibitory to *P. hysterothorus* at 75 DAS of sunflower and decreased with increase in stages of sunflower growth in the field. In pot experiments, sunflower rhizosphere soil was found most inhibitory at 75 DAS and less at 120 DAS. Here inverse trend of inhibition was observed in both sunflower rhizosphere soil and green manuring.

This type of research work is being done in developed countries to minimize or eliminate the use of the present hazardous herbicides and replacing them with eco-friendly herbicides based on natural plant products or allelochemicals. The generation of such information would help in; a) developing environmental friendly weed management practices to reduce the yield losses in field crops caused by the weeds and b) to overcome the problems associated with present herbicides.

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