

Allelopathic effects of sunflower (*Helianthus annuus*) on germination and growth of *Parthenium hysterophorus*

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ABSTRACT

Investigations were done to determine the effects of sunflower rhizosphere soil (SRS) in pot culture and of sunflower green manuring (SGM) in field studies at 75, 90,105 and 120 days after sowing (DAS) on the germination and growth of *Parthenium hysterophorus* weed. Two cover crops greengram [(GG) (*Vigna radiata*)] and Pearl millet [(PM) (*Pennisetum glaucum*)] were used. Field experiments revealed that all growth and yield attributes of *P. hysterophorus* were inhibited maximum at 75 DAS old SGM and in SGM + PM. The inhibitory effects of sunflower decreased at later stages. Likewise in Pot experiments, SRS at 75 DAS proved most inhibitory to growth and yield attributes of *P. hysterophorus* than at later stages. Allelochemicals were identified in sunflower crop by paper chromatography and spectroscopic analysis. These results suggested that sunflower green manuring and its rhizosphere soil has herbicidal potential and may be used for biological control of weeds for sustainable agriculture and to safeguard the environment.

Keywords: Allelochemicals, germination, greengram, Green manuring, growth, *Helianthus annuus*, *P. hysterophorus*, rhizosphere soil

INTRODUCTION

Parthenium hysterophorus (L.) Beauv. (bitter weed, congress grass) has become major invasive weed in India. It grows along the roadsides, around the agricultural fields and on wastelands. Its allelopathic nature is known, the water soluble phenolics and sesquiterpene lactones are found in roots, stems, leaves, inflorescences, pollens and seeds (9). The allelopathy indicates, where one plant exerts a detrimental effect on another through the production of germination and growth inhibiting substances (19,21). It can play important role in regulating the plant diversity (7). Due to the increasing numbers of herbicide-resistant weeds and environmental concerns due to the use of synthetic herbicides, considerable efforts are being done to develop alternative weed management strategies. Herbicides controls the weeds effectively but their extensive use has resulted in the development of resistant weeds (3,10) and contamination of agricultural produce and environment (5,8). Thus, interest in alternative methods to control *P. hysterophorus* weed has increased recently (2,18,22,25).

Allelopathic potential of sunflower for weed control has been reported in its various cultivars (Ramsum HS-52, Peredovik, Hybrids 201, 8941). In green house studies,

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sunflower 'Russian mammoth' reduces both the seed germination and biomass of weeds (11). Its aqueous extracts as well as growing plant inhibit the seed germination and seedling growth of *Abutilon theophrasti*, *Datura stramonium*, *Ipomoea* species and *Brassica kaber* (12,26). Its aqueous extracts also reduced the germination (36-56%) and seedling growth (22-57%) of *P. hysterophorus* and *Trianthema portulacastrum*, *Amaranthus viridis*. However, in field studies the germination (83-95%), growth (79-95%) and chlorophyll contents of these weeds were drastically reduced. Sunflower-oat rotation over 5 years period significantly lowered the density of grassy and broadleaf weeds in fields than in control plots (13). Although weed density increased in all plots over the five seasons, but 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 EPTC (S-thyidipropyl carbomothioate) herbicide was applied or not, clearly showing the efficacy of sunflower mediated weed control. In sunflower-wheat rotation field trials, sunflower decreased the density and dry weight of wild oat and *Cirsium arvense* weeds in the following wheat crop (6). Soil incorporation of sunflower residues significantly reduced the number of dicot weeds by 66% compared with control (1). In pot experiments, sunflower straw inhibited the plant height of wild oat, *Agropyron repens*, barnyard grass, *Ambrosia artemisifolia* and lambsquarter and decreased the biomass of last three weed species (16). Application of sunflower residues at 2 t/ha or its preceding crop reduces the population of *Cleome viscosa*, *Chorchorus trilocularia* and *Cyperus iria* (16).

The potential of sunflower as source of allelochemicals is well known (24) and bioassays of leaf aqueous extracts show strong inhibition in germination and root length of test plant species. The leaf aqueous extracts of sunflower 'SH 222' contained 5 new guaianolides and the annuolides possessing the allelopathic activity. All guaianolides were allelopathic to dicotyledon species and these are likely to be involved in the allelopathic activity of sunflower cultivars (15). Macias (15) characterized many new allelochemicals viz., 16 sesquiterpene lactones, 5 flavonoides, 4 kaurenoed diterpenes, 14 bisnorsesquiterpenes and sesquiterpene heliannuols in sunflower plant. The heliannuols inhibited the dicot weeds species, hence, may be excellent source as pre and post emergence herbicides at very low doses (10^{-4} to 10^{-9}).

This study aimed to determine (i). the potential of sunflower as natural herbicide to reduce the *P. hysterophorus* infestation and (ii). to develop environmental friendly weed management practices to reduce the yield losses in field crops caused by weeds.

MATERIALS AND METHODS

This study consisted of pot culture and field studies conducted at Haryana Agricultural University, Hisar, India during 2006-2007. Sunflower variety cv. Mahyco and Green gram (*Vigna radiata*, W.K.-851); (Pearlmillet), (*Pennisetum glaucum* Cr. HHB - 67) crops were used. 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. Sunflower was grown in field and was used for green manuring and its rhizosphere soil was used for field and pot experiments.

I. Pot experiment

This experiment consisted of two types of soil i.e. non-sunflower rhizosphere soil (control) and sunflower rhizosphere soil (SRS) and (*P. hysterophorus*) was test weed. The treatments were replicated thrice in a completely randomized design. The soil used in pot trial was sandy loam in texture, alkaline in pH (8.4) and poor in organic carbon (0.25%). Soil for control treatment was brought from the field where sunflower was not growing. The rhizosphere soil was brought from the sunflower plants rhizosphere upto 30 cm depth at 75, 90, 105 and 120 days after sowing (DAS) to determine its phytotoxicity against *P. hysterophorus* weed.

Immediately after sunflower crop harvest, the soil was dug, clods were crushed to powder and the soil was sieved through 2 mm-mesh sieve to remove all plant residues etc. All pots (25 cm dia) were lined with polythene sheets to prevent adsorption of allelochemicals by pots or leakage of leachates from the pots. The pots were filled with 4.0 kg soil/pot (DW) as per the treatments. Recommended doses of nitrogen and phosphorous were applied in pots and mix in soil. The pot soil was leveled and pressed with hand to remove air pockets and was irrigated with 900 ml tap water. Next day as per treatments, 5-seeds of test crop were sown (kept on soil surface) per pot. Immediately, the seeds were covered with 500 g dry sieved soil per pot, to prevent crust information over the germinating seeds. For test weeds, the soil infested with *P. hysterophorus* seeds was used. After the completion of germination, the pots were irrigated with tap water as required. To determine the phytotoxicity on particular crops and weeds other weeds were removed from the pots. The thinning of plants in pots was done at 15 days and 5-crop plants were kept per pot. 30 May, 15 June, 30 June 15 July and 30 July, 2005 at various stages of sunflower i.e. 75, 90, 105 and 120 DAS respectively. Harvesting of experiment was done on 15 October, 30 October, 15 November, November 30 November and 15 December, 2005. Same experiments were repeated again in 2007 and data of two year were pooled for analysis.

II. Field Experiment

The field experiment consisted of 3 factors (i). Age of sunflower at green manuring: 4(75,90,105,120 days), (ii). Green manure treatments: 2[control (without green manure), green manure] and (iii). Crops : 2(Pearmillet, greengram). The treatments were replicated 4 times in completely randomized design.

Sunflower plants were grown in 2001 and 2002 with recommended cultural practices in experimental field of Agronomy Farm. The field soil was loamy-sandy and medium in soil fertility (Nitrogen 66.1 kg/ha, Phosphorus 36.8 kg/ha and Potassium 344 kg/ha and pH 8.12). A basal dose of nitrogen and phosphorus 60 kg/ha was drilled at at crop sowing. The nitrogen applied to greengram and pearlmillet was 25 and 120 kgN/ha respectively, whereas, to *P. hysterophorus* weed no nitrogen was applied. After green manuring of sunflower, cover crops (*Vigna radiata*, W.K.-851; *Pennisetum glaucum* Cr. HHB-67) were sown. Sunflower plants were harvested for green manuring at 60, 75, 90, 105 and 120 days after sowing (DAS), the harvested sunflower plant biomass (96.0 q/ha) was chopped and incorporated into the soil as per treatments. The plots were irrigated and the chopped tissues of sunflower were mixed with soil up to 15 cm soil depth in April, 2001.

Plots were 2.5 m x 2 m and were separated by 30 cm buffer strips. The cover crops, green gram (*Vigna radiata*, W.K.-851) and pearl millet (*Pennisetum glaucum* Cr. HHB - 67) were sown in 25 cm wide rows. For *P. hysterophorus* seeds, soil from natural seed bank (natural community) was collected and sieved through 2 mm mesh to remove unwanted plant materials. Sieved soil of *P. hysterophorus* seeds was mixed in treatment plots. Three seeds each of green gram and pearl millet were sown manually per hill. No herbicides were applied in the experimental plots, but other weeds were manually removed from plots except *P. hysterophorus*. The plots were irrigated when required. At maturity, plant population, height, grain yield, plant population, tillering/branches/ number of capitulum/plant, biomass yield/ plant and per plot were measured using 1m² quadrat at three sample point per plot. Green manuring of sunflower in plots as per treatments was done on 15 June, 30 June 15 July and 30 July, 2005 at various stages of sunflower age i.e. 75, 90, 105 and 120 DAS respectively. Harvesting of experiment was done on 30 October, 15 November, 30 November and 15 December, 2005. Same experiments were repeated again in 2007 and data of two year were pooled for analysis.

III. Extraction and identification of allelochemicals in sunflower

Extraction of phenolic compounds in sunflower plants was done as under (14). Two hundred g biomass of sunflower plants was collected from the field and extracted with boiling methanol and filtered through Whatman's filter paper No. 1. The filtrate was then extracted with petroleum ether to eliminate chlorophyll. The methanolic extract was evaporated and analysed for total phenolics. One ml of the methanolic extract of sample was diluted to seven ml with distilled water. The contents were shaken thoroughly and 0.5 ml of Folin-Denis reagent was added. After, 3 min one ml of saturated solution of sodium carbonate was added and total volume was made 10 ml by adding distilled water. The samples were kept in dark. The absorbance was determined on spectrophotometer at 760 nm. The paper was developed in two solvent systems. Such as Butanol: Acetic acid: water (BuOH: CH₃COOH: H₂O) 63: 10:27, v/v) BAW and Butanol: Formic acid and water (BuOH: HCOOH: H₂O) 82: 1.5: 16.5, v/v) (BFW) in a descending manner. The R_f values of spots and their colour under Ultra-violet (U/v) light with and without ammonia exposure was noted. The identification of compounds were also done with colour reactions namely diazotized p-nitroaniline diazotised sulfanillic acid, ferric chloride-standards were also run in the same solvent systems for the confirmation and identification. Further confirmation of compounds in sunflower was done by separating the methanolic extract of sunflower by column chromatography and subjected to spectroscopic analysis viz. ultraviolet, infra-red, nuclear magnetic resonance and mass spectra etc.

Statistical analysis

The data was analysed statistically using ANOVA. 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, followed by separation of treatment means from the control at 0.05. level was used for comparisons of treatment.

RESULTS AND DISCUSSION

1. POT EXPERIMENTS

Plant Population: The sunflower rhizosphere soil (SRS) collected at 75, 90, 105 and 120 DAS decreased the plant population of *P. hysterophorus* than non-sunflower soil (control) (Table 1). The SRS at 60 DAS proved most inhibitory to *P. hysterophorus* (61.5% reduction). The magnitude of inhibition in plant population of *P. hysterophorus* followed the order SRS at 60 (61.5%) > 75 (48.5%) > 90 (28.4%) > 120 (17.6%) > 105 (0%) DAS (Table 1). At 105 DAS, the effect of SRS on plant population of *P. hysterophorus* was similar to non-sunflower rhizosphere soil.

Plant height: The sunflower rhizosphere soil (SRS) collected at 75, 90, 105 and 120 DAS decreased the height of *P. hysterophorus* plants than non-sunflower soil (control) (Table 1). The SRS at 60 DAS was most inhibitory to *P. hysterophorus* (65.8% reduction) height. The magnitude of inhibition in height of *P. hysterophorus* followed the order SRS at: 60 (65.8%) > 75 (55.5%) > 90 (41.4%) > 105 (38.8%) > 120 (18.7%) DAS (Table 1).

Number of branches/tillering: The SRS collected at all stages (60, 75, 90, 105 and 120 DAS) inhibited the branching in *P. hysterophorus* (Table 1). The magnitude of inhibition followed the order: 60 (48.8%) > 75 (48.5%) > 90 (28.5%) > 120 (11.1%) > 105 (6.2%) DAS. The results indicated that SRS at 120 DAS was least harmful to branching than earlier stages.

100-Seeds weight: SRS at 75 DAS proved most inhibitory to 100-seeds weight of *P. hysterophorus* (14.8%), but was less inhibitory (0.0%) at 105 DAS. The magnitude of inhibition to seed weight in *P. hysterophorus* followed the order 75 (14.8%) > 60 (8.05%) > 90 (7.6%) > 120 (4.0%) > 105 (0.0%) DAS (Table 1).

Seed yield: The inhibitory effects of rhizosphere soil to *Parthenium* seed yield followed the order: 60 (44.6%) > 75 (34.7%) > 90 (18.8%) > 120 (17.5%) > 105 (0.0%) DAS (Table 1). Except at 105 DAS, all stages of sunflower rhizosphere soil decreased the seed yield per plant of *P. hysterophorus*. Maximum harmful effect of rhizosphere soil in seed yield was observed at 60 DAS (44.6%). Whereas in per pot yield, the maximum decrease in rhizosphere soil in seed yield was observed at 60 DAS (59.9%). The inhibitory trend of rhizosphere soil on seed yield followed the order 60 (59.9%) > 75 (55.4%) > 105 (56.0%) > 90 (48.4%) > 120 (9.0%) DAS respectively (Table 1).

Biomass yield: The rhizosphere soil decreased the biomass yield per plant of *Parthenium* in following order : 60 (44.6%) > 75 (34.7%) > 90 (18.8%) > 120 (17.5%) > 105 (0.0%) DAS (Table 1). Whereas, inhibition in biomass yield per pot of *P. hysterophorus* was observed at 60 DAS (57.3%) (Table 1). The trend of harmful effects of rhizosphere soil on biomass yield followed the order 60 (57.3%) > 75 (40.6%) > 90 (35.4%) > 105 (23.4%) > 120 (13.7%) respectively (Table 1).

Table 1. The phytotoxicity effects of sunflower rhizosphere soil on growth and yield of *P. hysterophorus* in pot experiments

Treatment	Plant population	Plant height	No. of branches	100-seed wt (g)		Yield/Plant (g)		Yield/pot (g)	
				Grain	Straw	Grain	Straw	Grain	Straw
Control	7.8 a	82.3a	8.8a	0.25a	Sunflower rhizosphere soil_ 60 DAS		7.18a	2.18a	52.3a
Rhizosphere soil	3.0 b (-61.5)	28.1 b (-65.8)	4.5 b (-48.8)	0.23b (-8.0)	0.62 b (-44.6)	3.30 b (-54.0)	0.96 b (-59.9)	22.3 b (-57.3)	
Control	6.8 a	81a	9.15a	0.27a	Sunflower rhizosphere soil_ 75 DAS		7.15 a	2.58 a	49.0 a
Rhizosphere soil	3.5 b (-48.5)	36b (-55.5)	5.0 b (-45.0)	0.23 b (-14.8)	0.75 b (-34.7)	4.0 b (-43.6)	1.15 b (-55.4)	29.15 b (-40.6)	
Control	7.0a	82a	8.50 a	0.26 a	Sunflower rhizosphere soil_ 90 DAS		7.0 a	2.60 a	48.3 a
Rhizosphere soil	5.0 b (-28.5)	48 b (-41.4)	6.5 b (-23.5)	0.24 b (-7.6)	0.95 b (-18.1)	5.15 b (-27.1)	1.15 b (-48.4)	31.25 b (-35.4)	
Control	6.5 a	80a	8.0 a	0.24a	Sunflower rhizosphere soil_ 106 DAS		7.0a	2.60 a	43.2 a
Rhizosphere soil	6.5 a (0.0)	45 b (-38.8)	7.5 a (-6.2)	0.24 a (0.0)	1.0 a (0.0)	5.56 b (-21.4)	1.35 b (-56.0)	13.0 b (-23.4)	
Control	8.5a	81.3 a	9.0 a	0.25 a	Sunflower rhizosphere soil_ 120 DAS		7.85 a	2.29 a	45.3 a
Rhizosphere soil	7.0 b (-17.6)	66.0 b (-18.7)	8.0 b (-11.1)	0.24 b (-4.0)	1.10 b (-17.5)	6.10 b (-21.7)	2.0 b (-9.0)	39.0 b (-13.7)	

Different letters in a column represent significant difference at $P < 0.05$; DAS = Days after sowing; Data in parenthesis indicate % stimulation (+) and inhibition (-) over controlTable 2. Alkochemicals present in stem and leaves of sunflower (*Helianthus annuus*)

Phenolic acids	Solvent system				UV fluorescence				Physico-chemical Properties of compounds					
	Butanol acetic acid water (BAW)		Butanol formic acid water (BFW)		Without Ammonia Exposure		With Ammonia Exposure		<i>p</i> Nitro-aniline		Sulfanilic chloride		Hopphars Reaction	
	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves
Ferulic	0.44	0.46	0.43	4.44	Light blue	Light blue	Yellow green	Yellow green	Green	Purple	Blue	Blue	Red	Red
p-Coumaric	0.63	0.66	0.64	0.65	Light blue	Light blue	Yellow green	Yellow green	Blue	Light blue	Blue	Blue	Red	Red
Syringic	0.67	0.66	0.65	0.65	Light blue	Light blue	Yellow green	Yellow green	Blue	Red	Blue	Blue	Red	Red
Chlorogenic acid	0.59	0.58	0.44	0.44	Light blue	Light blue	Yellow green	Yellow green	Brown	Dark blue	Blue	Blue	Red	Red
Isochlorogenic acid	0.78	0.77			Light blue	Light blue	Yellow green	Yellow green	Brown	Dark yellow	Blue	Blue	Red	Red
Neochlorogenic acid	0.73	0.74	0.75	0.77	Light blue	Light blue	Yellow green	Yellow green	Brown	Dark yellow	Blue	Blue	Red	Red
Vanillic acid	0.93	0.95	0.9	0.91	Light blue	Light blue	Yellow green	Yellow green	Pink	Orange	Blue	Blue	Red	Red
p-Hydroxybenzoic acid	0.87	0.88			Light blue	Light blue	Yellow green	Yellow green	Blue	Yellow	Blue	Blue	Red	Red
Caffeoylquinic acid	0.8	0.82	0.83	0.85	Light blue	Light blue	Yellow green	Yellow green	Blue green	Dark yellow	Blue	Blue	Red	Red
Unidentified phytochemicals	0.52	0.6			Light blue	Light blue	Yellow green	Yellow green	Brown	Dark yellow	Blue	Blue	Red	Red

Sunflower rhizosphere soil reduces the seedling growth (population, plant height) and yield attributes (seed and biomass) of *P. hysterophorus* and *Trianthema portulacastrum* weeds in pot experiments (18). The effect of such soil was due to the presence of allelochemicals (p-hydroxybenzoic acid, vanillic acid, caffeic acid, ferulic acid) released by sunflower roots in soil. Pariana (17) recommended that growing of sunflower on large scale may not be harmful to the crops provided (i). there is enough fallow period after the sunflower crop harvest and sowing of next crop and (ii). no residue of sunflower were allowed to decompose in the soil. The allelopathic compounds released by sunflower in the soil adversely affects the succeeding crops upto 12-weeks (20). The phytotoxicity of soil incorporated residues decreased the height and dry matter of pearl millet and soybean upto 120 days. Sandhu (20) also suggested that the adverse effect of sunflower decreased with delay in sowing. It was conspicuous upto 4-weeks after incorporation. The additional application of nitrogen did not overcome the adverse effects of sunflower. It has been also stated that when cotton was sown 3.8 weeks after leaf litter incorporation, germination was not reduced and dry matter was increased. The analysis of soil at different intervals after incorporation of various plant parts of sunflower revealed that sunflower roots were the major source of phenolic compounds in the soil and their release continued for 6-7 weeks after the incorporation of the plants parts (20).

II. FIELD EXPERIMENTS

Plant population: The phytotoxicity of sunflower green manure to plant population of *P. hysterophorus* at 75 DAS was highest than green manure at later stages (viz., 90, 105 and 120 DAS). In GM (Green manuring) + PM (Pearlmillet), the population of *P. hysterophorus* was completely inhibited (100%) (Figure 1) in all stages of sunflower green manuring as compared to control plots. Plant population of *P. hysterophorus* in GM + GG (Greengram) at 75 DAS was reduced 100% i.e. completely controlled, however at 90, 105 and 120 DAS showed significant reduction and followed the order 75.0 > 62.5 > 62.5%, respectively.

Plant height: Green manuring of sunflower at all stages without cover crops (GG + PM) significantly reduced the plant height of *P. hysterophorus* as compared to control (Figure 1). The results revealed that GM + PM, provided 100% control of *P. hysterophorus*. In GG green manuring at 75 DAS provided 100% reduction in Plant height of *P. hysterophorus*, however, at 90 DAS and 120 DAS the reduction in plant height was identical (63.1%), whereas, at 105 DAS, the effect become less inhibitory (57.8%) (Figure 1).

Number of branches: The Branches of *P. hysterophorus* were completely absent (100% inhibition) under GM + PM and GM + GG in all stages of sunflower green manuring as compared to control plots (Figure 1). The results indicate that branches of *P. hysterophorus* were drastically reduced by pearl millet and green gram 68.4% and 52.6%, respectively, without green manuring than pure *Parthenium* (Figure 1). Green manuring + PM or GG provided 100% reduction in branching in *P. hysterophorus*.

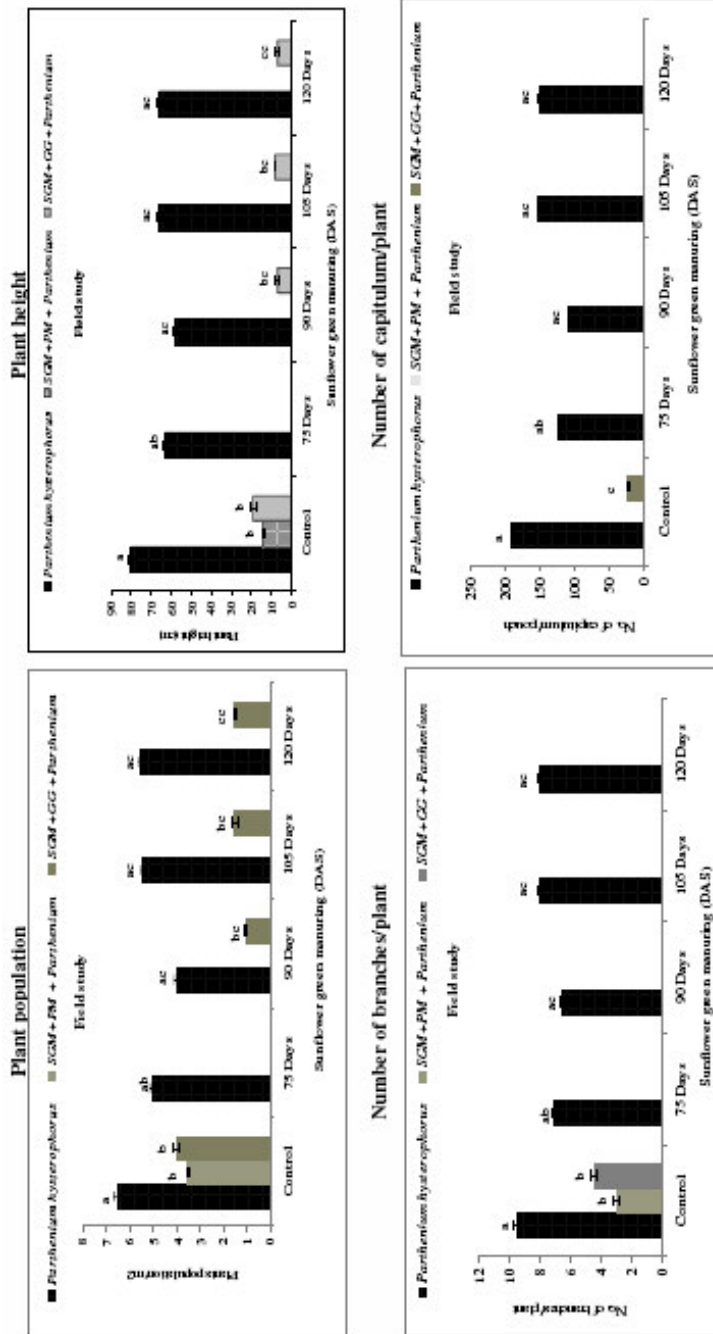


Figure 1. Effects of sunflower green manuring in field studies at different growth stages on plant population, plant height, number of branches/plant and number of capitulum/pouch of *P. hysterophorus*. Different letters in a figure represent significant difference at $P < 0.05$. SGM: Sunflower green manuring; PM: Pearl millet (*Pennisetum glaucum*); GG: Green gram (*Vigna radiata*).

Number of capitulum: Compared with control, capitulum of *P. hysterophorus* was reduced 100% under GM + PM and GM + GG (Figure 1) at all stages of sunflower green manuring. Even without green manuring of sunflower, capitulum in *P. hysterophorus* was 100% absent with pearl millet, whereas, green gram significantly reduced the number of capitulum 88.3% than pure parthenium (Figure 1).

Seed yield: Similarly to capitulum, 100% reduction in seed yield of *P. hysterophorus* was found under GM + PM and GM + GG at all stages of sunflower green manuring viz., 75, 90, 105 and 120 DAS (Figure 2). In GG, green manuring at 105 DAS caused 62% reduction in seed yield/plant of *P. hysterophorus*. The results indicated that without green manuring of sunflower, seed yield in *P. hysterophorus* was nil (zero) with pearl millet, whereas, green gram drastically reduced the seed yield by (74.3 %) than pure parthenium (Figure 2).

Biomass yield: Like the seed yield, the biomass yield of *P. hysterophorus* was nil (zero) under GM+PM at all stages of sunflower green manuring (75, 90, 105 and 120 DAS) (Figure 2). In GG, green manuring at 75 and 105 DAS also provided 100% reduction in biomass yield of *P. hysterophorus*, whereas, at 90 and 120 DAS the inhibitory effect was 85.2% and 22.3 %, respectively, than control (Figure 2). Azania (2) and Ben-Hammouda *et al.* (4) reported that sunflower Cv. MSFH-I and BSH-I reduced the weed population and weed biomass in field conditions. Weed population of *Flaveria australasica*, *P. hysterophorus*, *Amaranthus viridis*, *Trianthema portulacastrum* and *Portulaca oleracea* were reduced by 83,84,85,91 and 85% respectively. The weed biomass was reduced by 94,93,90,79 and 92% in *Trianthema portulacastrum*, *P. hysterophorus* L, *Flavaria australasica* Hook, *Portulaca oleracea* and *Amaranthus viridis* respectively. Leather (12) investigated that surface incorporation of mature sunflower at 320 quintal/ha (fresh weight) provided > 86% reduction in population of *Sida spinosa*, *Digitaria sanguinalis* and *Amaranthus albus* weeds. Singh (22) studied the herbicidal potential of sunflower on *P. hysterophorus* and *Trianthema portulacastrum* in pearl millet and green gram and fallow plots in field and found 100% control of *P. hysterophorus* in pearl millet plots.

III. Identification of allelochemicals from sunflower

The allelopathic potential of sunflower raised the interest of identifying the substances responsible for the allelopathic action on other plants. Ten allelochemicals have been identified in this study from different parts of sunflower (Table 2). The Rf values of compounds from paper chromatographs and their physico-chemical properties are given in (Table 2). The result revealed that the sunflower shoots and leaves contained nine compounds, the Rf values of 0.44, 0.46 and 0.43, 0.44 correspond to Ferulic acid in BAW and BFW solvent system respectively in sunflower. Similarly, the Rf values of 0.63, 0.66 and 0.64, 0.65 corresponded to P-caumaric in BAW and BFW system respectively in shoots and leaves. Likewise, the Rf values 0.67, 0.66 and 0.65, 0.65 and 0.59, 0.44 and 0.44 corresponded to Syringic and Chlorogenic acid in BAW and BFW system respectively. Whereas in BAW the Rf values 0.78 and 0.77 corresponded to Isochlorogenic acid in stem and leaves respectively in sunflower. The Rf values 0.74, 0.74 and 0.75, 0.77 and 0.93, 0.95, 0.91 correspond to neochlorogenic acid and vanillic acid in BAW and

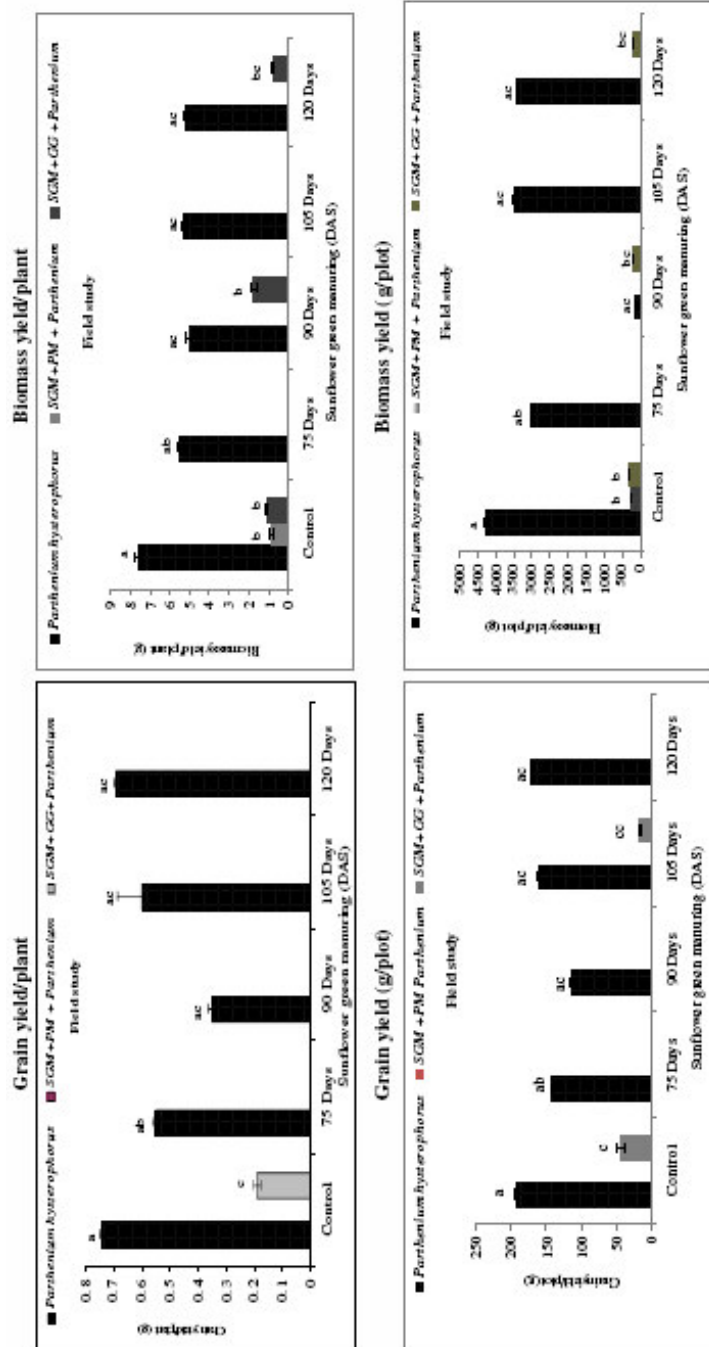


Figure 2. Effect of sunflower green manuring at different stages in field experiment on grain yield/plant, biomass yield/plot and biomass yield/plot of *P. hysterophorus*. Different letters in a figure represent significant difference at $P < 0.05$. SGM: Sunflower green manuring; PM: Pearl millet (*Pennisetum glaucum*); GG: Green gram (*Vigna radiata*).

BAW system in respectively in stems and leaves. Whereas the Rf values 0.87 and 0.88 were observed only in BAW system correspond to P-hydroxybenzoic acid in sunflower stem and leaves respectively. Likewise, the Rf values 0.80, 0.82 and 0.83, 0.85 correspond to caffeoylquinic acid in BAW and BFW system respectively in shoots and leaves. Unidentified compounds with observed Rf values 0.52 and 0.60 only in BAW system. All Rf values observed under UV light and gave their respective colour without exposure to ammonia vapour and gave their respective colour. For confirmation of the compounds, the compounds were sprayed with diazotized-P-nitro aniline, sulfanilic acid, ferric chloride-potassium ferricyanide and also tested with Hoepfner's coplin reaction. All compounds gave their respective colour for their identification with each indicator (Table 2). Chemical studies on different parts of *Helianthus annuus* have led to the identification of a number of compounds; prominent among them being the sesquiterpene lactones. Spring et al. (23) isolated two sesquiterpene lactones named niveusin C and 15-hydroxy-3-dehydroxy fruticin from leaves and stems of *Helianthus annuus*. Both sesquiterpene lactones strongly inhibit, indole-3-acetic acid (IAA) induced elongation growth of *Avena sativa* L. coleoptile segments and *Helianthus annuus* L. hypocotyl 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 (27). Spring and Benz (24) later identified six sesquiterpene lactones by HPLC separations from capitate glandular trichomes of *Helianthus annuus* L. Pariana (17) 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. Compound A impairs the respiratory machinery of the germinating propagules, their impact depends on the time and concentration. There has been a general understanding among the warring weed scientists 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.

CONCLUSIONS

The green manuring of sunflower and its rhizosphere soil has potential to reduce the seed germination, plant population, number of capitulum and biomass of *P. hysterophorus*. Sunflower Green manuring at various growth stages with pearl millet cover crop proved most inhibitory to parthenium. The green manuring of sunflower at 75 days was more inhibitory to *P. hysterophorus* and magnitude of inhibition decreased with increase in sunflower age. In pot experiments, sunflower rhizosphere soil was found most inhibitory to *P. hysterophorus* growth and development at 75 DAS.

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