https://doi.org/10.26651/2017-42-1-1104 0971-4693/94 Euro 20.00
Allelopathy Journal 42 (1): 37-48 (September, 2017) *International Allelopathy Foundation* 2017
Table: 4, Figs : 2

Allelopathic effects of sunflower on succeeding mungbean (*Vigna radiata* L. Wilczek) crop

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(Received in revised form: July 13, 2017)

ABSTRACT

We studied the allelopathic effects of sunflower on the succeeding mungbean crop and also identified and quantified the allelochemicals present in different plant parts of sunflower using HPLC. Phenolic compounds present in leaves of sunflower were : Chlorogenic acid (18.53±0.66 mg/100 g) > trans-ferulic acid (17.96 ±1.15 mg/100 g) > caffeic acid (17.32±1.59 mg/100 g) > vanillic acid (15.34±1.07 mg/100 g), while stem contained trans-ferulic acid (17.92±1.57 mg/100 g) > chlorogenic acid (16.31±0.95 mg/100 g) > vanillic acid (15.15±0.91 mg/100 g) and root contains only trans-ferulic acid (13.6±1.2 mg/100 g). The seed yield of mungbean was significantly higher in fallow-mungbean rotation (716 kg/ha) than in sunflower-mungbean sequence (593 kg/ha). Among the sowing periods, the crop sown on June 14 gave significantly higher seed yield (904 kg ha^{-1}) than other sowing dates except June 21 (837 kg ha^{-1}). The seed yield of mungbean in fallow-mungbean rotation sown on June 14 was 1038 kg ha^{-1} than sunflower-mungbean rotation sown on June 14 (770 kg ha^{-1}).

Key words: Allelochemicals, field study, *Helianthus annuus*, HPLC, laboratory, mungbean, phenolic compounds, seed yield, sunflower, *Vigna radiata*,

INTRODUCTION

Allelopathy plays an important role in the agroecosystems, leading to various interactions between the crop-crop, crop-weed and tree-crops. Generally, these interactions are harmful to the receiver plants but provide a selective benefit to the donor. Soil microbes determines such interactions as they change the allelochemicals and nature of allelopathic interactions. The allelochemicals released from the plant residues are left in fields after crop harvest.

Sunflower (*Helianthus annuus* L.) is source of high quality vegetable oil and exhibits allelopathic effects on subsequent crops and weeds (23,29). It is important oil seed crop in Karnataka due to its day neutral nature and short duration (3). Sunflower is thermo and photo-insensitive, hence it can be grown round the year in sub-tropics, hence, fits well in the multiple cropping systems. However, yield of some crops following sunflower are lower than normal, possibly because of allelopathic inhibition. In sunflower several substances (phenolic compounds, diterpenes and triterpenes) with allelopathic properties have been isolated and chemically characterised (24,25,26). More than 200 allelopathic compounds have been isolated from different cultivars of sunflower. In many allelopathic

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studies indicated that extracts and leachates of residues or plant parts are added to the soil for assessing their allelopathic potential (20).

Allelochemicals released from the crops and weeds *etc* or their residues are phytotoxic to neighbouring plants and sometimes their own plants. The bioactive concentration of allelochemicals in soil depends on the sorption, fixation, leaching, chemical and microbial degradation (8). Sunflower is allelopathic to succeeding crops (11,14,29,30,31). The mungbean or greengram (*Vigna radiata* L. Wilczek) is major pulse crop in India.

This study aimed to (i). Identify and quantify the allelochemicals present in different plant parts of sunflower, (ii). residual/allelopathic effects of sunflower on succeeding mungbean crop and (iii). find optimum fallow duration after the harvest of sunflower and between sowing of mungbean to minimise the harmful allelopathic effects.

MATERIALS AND METHODS

This study consisting of field study, laboratory study and chemical analysis was done during 2014. The study consisted of field study and chemical analysis studies.

Field study

It was done in summer -2014 at Dharwad (5°26' N latitude, 75°01' E longitude and altitude; 678 m above mean sea level) The soil of experimental site was medium black clay soil, low in nitrogen (253.4 kg ha^{-1}), medium in phosphorous (27.7 kg ha^{-1}) and rich in potash (325.84 kg ha^{-1}). Preceding crop was sunflower (grown from March to June 7, 2014). Experiments details were as under:-

Experiment was laid out in a split plot design and treatments were replicated thrice.

(i). **Main plot:** The rotation was in main plot (a). Fallow-mungbean and (b) Sunflower-mungbean

(ii)**. Sub-Plot:** Sowing dates of succeeding mungbean after sunflower harvest (June 14, 21, 28, July 5,12,19).

Main plot size was 24.1 m × 3.0 m (64.8 m^2) and sub plot size was 3.6 m × 3.0 m (10.8 m^2). Mungbean crop was sown at weekly intervals (14, 21, 28 June, 05, 12 and 19 July), row spacing (30 cm × 10 cm), seed rate (12 kg ha^{-1}), fertilizer applied (25:50:0 kg N, P_2O_5 and K_2O ha^{-1}) and harvested at full maturity (3, 8, 15, 20, 29 September and 3 October). The annual rainfall received during 2014 was 962.20 mm distributed in 69 rainy days. The total rainfall received during the summer mungbean crop period (June-October, 2014) was 633.5 mm distributed in 49 rainy days. The mean monthly maximum temperature ranged from 27.0^oC (July) to 30^oC (October). Whereas, mean monthly minimum temperature ranged from 19^oC (October) to 21.6^oC (June). At harvest, 5-plants per plot were sampled, oven dried at 70^oC (48 h) for complete drying, then pods were picked from the plants, counted and threshed for seeds. Seeds were cleaned and weighed for grain yield $plant^{-1}$.

Chemical analysis

Sunflower sample collection: Mature sunflower plants were harvested from the field and transferred to Laboratory. In laboratory these plants were partitioned into leaves, stem and

roots. They were dried (70 ^{o}C) for 24 hours and powdered and kept in polythene bag and stored in refrigerator.

Extraction of phenolic compounds

One g biomass of test samples was soaked in 100 ml hot distilled water (70-80^{o}C) acidified with 1 ml of acetic acid. The mixture was heated gently, mixed thoroughly using ultrasonic apparatus to exclude air bubbles from the residues and allowed to stand for 4 hours. The mixture of each sample was filtered through filter paper under vacuum and kept in refrigerator.

The reference standard compounds acid- chlorogenic acid, trans-p-coumaric acid, caffeic acid, trans-ferulic acid and chlorogenic acid were purchased from Sigma Aldrich (Aldrich Chemical Co., India). Solvents- Acetonitrile and acetic acid were purchased from Rankem (Rankem Co. Ltd., India).

Preparation of standard solutions: Standard stock solutions were prepared in methanol at 1 mg/ml concentration and stored in refrigerator at 5°C until use. All stock solutions were further diluted 10 times with methanol before injection. These stock solutions were stored in light resistant containers. In addition, five standard stock solutions (100 ppm) of 1 ml each were combined into a 10 ml volumetric flask at concentration of 14.30 ppm before HPLC injection.

HPLC analysis/ Apparatus:

The HPLC system included a Shimadzu LC 20 AT- VP solvent delivery system (pump), a SPD – 20 AVP UV/visible detector and a 7725 irheodyne injector with a 20µL loop volume. The class VP 6.01 data station software was utilized for integration. Separation was achieved using a Princeton C18 column (250 X 4.6 mm, Luna 5µ ID), (Merck, India). Binary gradient elution was used for the analysis, with the mobile phases of acetonitrile (solvent A) and aqueous 1 % acetic acid (solvent B), as follows: 100 % solvent B at 0 min; 85% solvent B at 12 min; 50 % solvent B at 20 min; 0 % solvent B at 22 min; 100 % solvent B at 24 min; isocratic elution of 100 % B, 24–30 min. The flow rate was 0.8 ml/min, with detection at 280 nm (17,18).

Retention times of these standard compounds and their major peaks in the extract were recorded. Chromatogram corresponding to plant sample of sunflower showed the good resolution and separation of the chromatographic peaks corresponding to the absorption at 280 nm can be seen.

Five phenolic compounds: Vanillic, trans-p-coumaric, trans-ferulic, caffeic and chlorogenic acids were used in the plant sample of sunflower. Both the analysis time for determination of all phenolic acids (< 30 min) and the profiles of the phenolic acids were found in agreement with those reported by others (6,17). Unidentified peaks observed during HPLC analysis indicate that other phenolic compounds might be synthesized by sunflower.

Quantitative analysis of phenolic compounds:

The phenolic compounds content was calculated using the following equation-

Phenolic content (mg/100 g of plant sample, mg/ml for extract) =

$$= \frac{\text{PA (sample)}}{\text{PA (standard)}} \text{ X } \text{Concentration of standards} \text{ X } \text{Volume of extract} \text{ X } \text{Dilution Factor (DF)} \text{ X } \frac{1}{\text{Weight of sample}}$$

Where, PA : Peak Area, Concentration of standards : 1 mg/10 ml (100 ppm), Volume of extract : 20 ml, Dilution factor (DF) : 10 (1 ml sample extract + 9 ml methanol), Weight of sample : plant samples weight (100 g).

Statistical Analysis: The data was statistically analysis using analysis of variance and means were compared using critical difference at the 5 % probability (15).

RESULTS AND DISCUSSION

Field study

Mungbean in fallow-mungbean rotation recorded higher seed yield (714 kg ha^{-1}) than in sunflower-mungbean sequence (593 kg ha^{-1}). The seed yield in fallow-mungbean was 16.94 % higher than in sunflower-mungbean sequence. This increase was due to increase in the yield parameters of mungbean in fallow-mungbean which resulted in higher number of pods (13.31) and higher number of seeds pod^{-1} (11.07) than sunflower-mungbean sequence (Table 1 and Fig. 1). Yield is the net result of various interactions *i.e.*, soil characters, weather parameters, inter and intra plant competition and various metabolic and biochemical interactions taking place throughout the plant growth. Reduction in the sunflower-mungbean sequence was mainly due to allelopathic effects of sunflower on succeeding mungbean crop. Sunflower releases the allelopathic compounds in soil, which affects the acceptor plants and affects many physiological processes and that these changes, being mostly statistically significant, showed a dose and time dependent relation (11,14).

Table 1. Yield and yield attributing characters of mungbean as influenced by different cropping systems and sowing periods

Sowing Dates	**Number of pod/ plant**			**Seed yield (kg/ha)**		
	S-M	**F-M**	**Mean**	**S-M**	**F-M**	**Mean**
June 14 (7*)	13.93	17.11	15.52	770	1038	904
June 21 (14*)	13.23	16.72	14.98	731	944	837
June 28 (21*)	11.03	13.84	12.44	660	793	726
July 05 (28*)	10.11	11.78	10.94	564	631	598
July 12 (35*)	9.78	10.26	10.02	430	461	446
July 19 (42*)	9.72	10.15	9.94	412	428	420
Mean	11.30	13.31		593	716	
For comparing means of	**S.Em±**	**CD (P = 0.05)**		**S.Em±**	**CD (P = 0.05)**	
Cropping system (C)	0.13	0.80		20.1	120.4	
Sowing period (D)	0.17	0.51		23.03	69.11	
Interaction- (CS x D)	0.25	0.73		31.2	95.47	

*Days after sunflower harvest, Dates of Sunflower harvest : June 7, 2014, FM **:** Fallow-Mungbean, SM : Sunflower-Mungbean

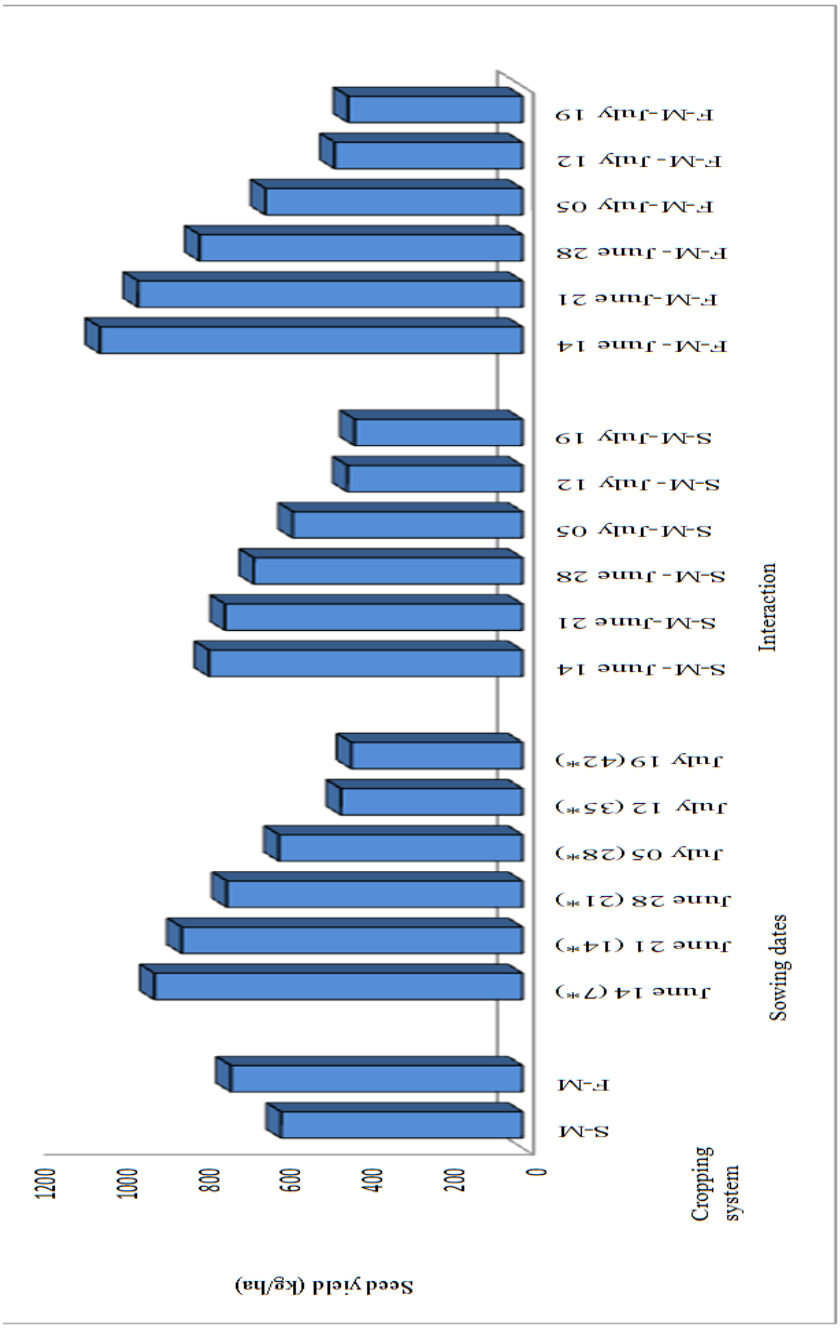

Figure 1. Effects of dates of mungbean sowing after sunflower harvest on mungbean seed yield. *Days after sunflower harvest, Dates of Sunflower harvest : June 7, 2014, FM : Fallow-Mungbean, SM : Sunflower-Mungbean

The yield parameters were directly correlated with the growth parameters. Mungbean sown in fallow-mungbean had higher number of branches per plant (8.78), LAI (2.76) and dry matter production (9.48 g plant^{-1}) than mungbean in sunflower-mungbean sequence (8.36, 2.58 and 8.96 g plant^{-1}, respectively). This was mainly due to the allelochemicals released from the sunflower residue in the rhizosphere soil either by volatilization, leaching and decomposition of sunflower plant residues (7,11,12,19,21). These allelochemicals might have inhibited the germination and growth parameters of mungbean, probably by affecting the cell division and elongation process that were very important at this stage or by interfering with enzymes involved in the mobilization of nutrients necessary for germination (4,5). They also may have decreased the carbon absorption and suppressed the photosynthesis, which stunted the growth of mungbean. These results corroborated the findings of Morris and Parish (28); Purvis and Jones (33), they reported that when sunflower residues were mixed into the soil, yield of wheat was decreased than without sunflower residues. The inhibitory effects of sunflower allelochemicals on seed germination appears to be mediated through a disruption of cellular metabolism rather than through organelle damage. Reserve mobilisation seems to be blocked or delayed during allelopatic stress. Disorder in respiration rate (O_2 uptake) leads to a limitation on the availability of metabolic energy (ATP level) and in consequence decreases seed germination and seedlings growth (12,20,22).

The crop sown on June 14 gave significantly higher seed yield (904 kg ha^{-1}) over other sowing periods except June 21 (837 kg ha^{-1}) with which it was on par. The crop sown on June14 registered 8, 19.62, 33.84, 50.66 and 53.53 % higher seed yield over other dates of sowing *ie.*, June 21, 28, July 5, 12 and 19, respectively (Table 1 and Fig. 1). The higher seed yield obtained in early sown crop was attributed to higher soil moisture during cropping period due to 242.20 mm rainfall during July. This coincided with the flowering and pod formation stage of early sown crop. The growth parameters (leaf area index and dry matter production plant^{-1}) were also significantly higher in early sown crop, which improved the yield components and ultimately the seed yield ha^{-1}. This was mainly due to the higher leaf area index at 60 DAS in early sown crop (3.04) than the crops sown after June 21, 28, July 5, 12 and 19 (2.96, 2.76, 2.52, 2.40 and 2.34, respectively) (Table 2). Thus, the higher values of LAI in early sown crop resulted in increased production of photosynthates contributing to higher dry matter production. The dry matter at harvest in early sown crop was 10.41 g plant^{-1} which was significantly higher than crop sown after June 21, 28, July 5, 12 and 19 (10.21, 9.42, 8.96, 8.36 and 7.97, respectively). This might be due to allelopathic agents influence the plant growth through the influenced on following physiological processes *viz.*, cell division and cell elongation, phytohormal induced growth, membrane permeability, mineral uptake, availability of soil phosphorus and potash, gas exchange, process of photosynthesis, respiration, stimulation or inhibition of certain specific enzymes, corking and clogging of xylem elements, conductance of water through stem interior water relationships (2,4,22,27,35,36).

Chemical analysis

Phenolic compounds present in different plant parts of sunflower were detected by HPLC chromatogram (Figure 1). The retention times of peaks in the HPLC chromatogram of the samples studied were compared with that of standards to identify the unknown plant

sample. Five standards were identified through their retention times and their UV spectra as compared to those of the standards (Fig. 2) and their concentrations were calculated by interpolation of the peak area as on the chromatograms of the HPLCs to a standard curve constructed using the peak areas of the authentic phenolic acids. As per literature, hot water extracts of stem and leaves of sunflower plants found superior over other methods and also that separation by thin layer chromatography is better than paper chromatography (1).

Separation of standard mixture of phenolic compounds by HPLC (280 nm) was found that at different retention time standard phenolic compounds separated were: 6.610= Vanillic acid, 6.800=Caffeic acid, 7.229= Trans-ferulic acid, 7.415= Trans-p-coumaric acid and 11.818=Chlorogenic acid (Table 3 and Fig. 2).

Table 2. Growth parameters of mungbean as influenced by different cropping systems and sowing periods

Sowing Dates	**Leaf area index (LAI at 60 DAS)**			**Dry matter (g plant $^{-1}$)**			**Number of branches/ plant**		
	S-M	**F-M**	**Mean**	**S-M**	**F-M**	**Mean**	**S-M**	**F-M**	**Mean**
June 14 (7*)	2.91	3.18	3.04	9.90	10.91	10.41	8.90	9.43	9.17
June 21 (14*)	2.83	3.10	2.96	9.72	10.69	10.21	8.76	9.30	9.03
June 28 (21*)	2.62	2.89	2.76	9.22	9.62	9.42	8.54	9.03	8.79
July 05 (28*)	2.42	2.62	2.52	8.79	9.12	8.96	8.16	8.82	8.49
July 12 (35*)	2.36	2.43	2.40	8.20	8.53	8.36	8.00	8.27	8.13
July 19 (42*)	2.33	2.36	2.34	7.94	8.00	7.97	7.77	7.83	7.80
Mean	2.58	2.76		8.96	9.48		8.36	8.78	
For comparing means of	**S.Em±**	**CD (P = 0.05)**		**S.Em±**	**CD (P = 0.05)**		**S.Em±**	**CD (P = 0.05)**	
Cropping system (C)	0.007	0.041		0.031	0.194		0.018	0.111	
Sowing period (D)	0.027	0.080		0.072	0.206		0.043	0.128	
Interaction- (CS x D)	0.038	0.112		0.106	0.288		0.061	0.181	

*Days after sunflower harvest, Dates of Sunflower harvest : June 7, 2014, FM **:** Fallow-Mungbean, SM : Sunflower-Mungbean

Table 3. Peak table of phenolic compounds (Standards)

Peak	Peak name	Retention Time	Area	Height	Area %	Height %
1.	Vanillic acid	6.610	2159840	351499	10.091	10.192
2.	Caffeic acid	6.800	4195578	710578	16.151	17.245
3.	Trans-ferulic acid	7.229	5237645	918801	24.612	25.125
4.	Trans-coumaric acid	7.415	4414062	741532	20.403	19.120
5.	Chlorogenic acid	11.818	5846677	1320941	26.305	27.110
6.	Un-identified	13.625	97839	14045	2.438	1.208
Total			22880226	4063298	100.000	100.000

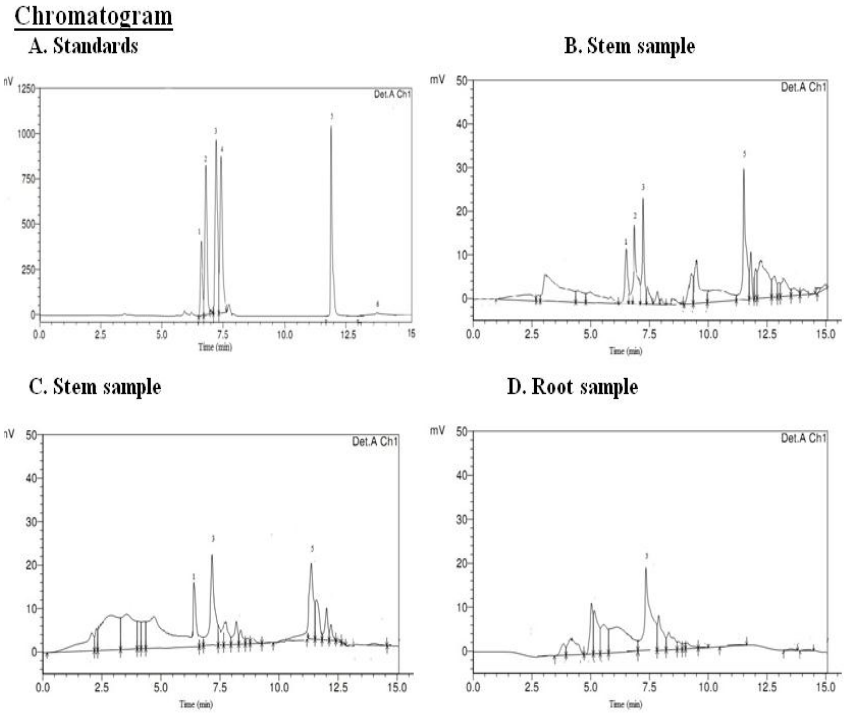

Figure 2: Chromatographic profile of phenolic compounds for standards (A) and plant samples (leaf, stem, root) of sunflower (B,C,D) and Phenolic compounds (1,Vanillic acid; 2, Caffeic acid ; 3, Trans- ferulic acid; 4, Trans-p-coumaric acid; 5, Chlorogenic acid; 6,Un identified;)

HPLC chromatogram for hot water extract of different plant parts of sunflower: Phenolic compounds present in the leaf of sunflower were separated at different retention times (6.432= Vanillic acid, 6.92=Caffeic acid, 7.19= Trans- ferulic acid, Not detected= Trans-p-coumaric acid and 11.73= Chlorogenic acid), where as phenolic compounds present in stem of sunflower were separated at different retention times (6.682= Vanillic acid, Not detected= Caffeic acid, 7.287= Trans- ferulic acid, Not detected= Trans-p-coumaric acid and 11.459= Chlorogenic acid). Phenolic compounds present in root of sunflower were separated at different retention time; it was found that only trans- ferulic acid was detected at retention time of 7.21 minute and remaining other compounds were not detected in chromatogram. By comparing the standard retention time, the identification of phenolic compounds was made (Table 4 and Fig. 2). The similar results were observed by Ibrahim *et al.,* 2011 and they reported that chromatographic analyses by HPLC revealed the presence of 13 secondary metabolites in residues of tested sunflower genotypes. All isolated compounds appeared to be phenolic, with exception of terpinol, which is a terpenoid derivative. The total concentration of phytotoxins (phenolic

compounds) was higher in most-suppressive potential genotypes compared to least suppressive genotypes (13,19).

Table 4. The phenolic compounds contents present in different plant parts of sunflower.

Peak	Peak name	Retention Time	Peak area of sample	Peak area of standard	Phenolic content (mg/100 g of plant sample)
	Leaf sample				
1.	Vanilic acid	6.432	165631	2159840	15.34 ± 1.07
2.	Caffeic acid	6.917	376710	4195578	17.32 ± 1.59
3.	Trans-ferulic acid	7.196	453602	5237645	17.96 ± 1.15
4.	Trans-coumaric acid	ND	-	4414062	-
5.	Chlorogenic acid	11.729	541764	5846677	18.53 ±0.66
Total		-	-	-	69.15
	Stem sample				
1.	Vanilic acid	6.682	163625	2159840	15.15 ± 0.91
2.	Caffeic acid	ND	-	4195578	-
3.	Trans-ferulic acid	7.287	469365	5237645	17.92 ± 1.57
4.	Trans-coumaric acid	ND	-	4414062	-
5.	Chlorogenic acid	11.359	476710	5846677	16.31 ± 0.95
Total		-	-	-	49.38
	Root sample				
1.	Vanilic acid	ND	-	2159840	-
2.	Caffeic acid		-	4195578	-
3.	Trans-ferulic acid	7.208	356135	5237645	13.6
4.	Trans-coumaric acid	ND	-	4414062	-
5.	Chlorogenic acid	ND	-	5846677	-
Total		-	-	-	13.6
Grand Total		-	-	-	132.13

ND: Not Detected

The results indicated that among five phenolic compounds, leaves contains 4-phenolic compounds [chlorogenic acid (18.53±0.66 mg/100 g) > trans-ferulic acid (17.96 ±1.15 mg/100 g) > caffeic acid (17.32±1.59 mg/100 g) > vanillic acid (15.34±1.07 mg/100 g)], stem contains 3-phenolic compounds [trans-ferulic acid (17.92±1.57 mg/100 g) > chlorogenic acid (16.31±0.95 mg/100 g) > vanillic acid (15.15±0.91 mg/100 g)] and root contains only one trans-ferulic acid (13.6±1.2 mg/100 g) (Table 4). Chemical analyses using HPLC indicated the presence of several phenolic compounds in water extract of residues from sunflower genotypes. These allelochemicals suppress the germination and seedling growth of succeeding crops *viz.*, wheat, mustard, mungbean, blackgram, soybean, sorghum and pearl millet and amaranthus weeds (2,9,10,11,12,16,21,34). These phenolic acids inhibits the ion uptake, chlorophyll biosynthesis, cell membrane stability, protein and hormone biosynthesis, cell division and changes ultra-structural components of cells (32).

The total quantity of phenolic compounds present in leaves of sunflower was 69.15 mg/100 g, whereas in stem and root it was 49.38 and 13.56 mg/100 g, respectively. Total phenolic content of the sunflower plant parts was 132.12 mg/100 g of plant material (Table 4). The results agree with findings of Macias *et al.* (1999a) and they reported that

heliannuol sesquiterpenoids isolated from the extract of cultivated sunflower (*Helianthus annuus* SH-222) were involved in allelopathic action of sunflowers against dicotyledons (26).

Several phytotoxic substances inhibitory to seed germination and seedling growth have been isolated from plant tissues and soils. These substances are collectively known as allelochemicals. They are usually secondary plant products or waste products of main metabolic path ways of plants and are released from plants as exudates, volatilizations, above ground leaching or decomposition of plant material (4). The important allelochemicals include alkaloids, terpenoids, flavonoids, steroids, tannins and phenols that usually have inhibitory effects on crops (27). Among these, phenolic compounds are perhaps the most commonly investigated and often constitute the principal allelopathic agents in allelopathic plants (6,8).

CONCLUSIONS

The phenolic compounds detected in hot water extraction of plant materials were the major allelochemicals present in different parts of sunflower. The hot water extraction of plant materials proved most effective extraction method with highest content of phenolic compounds. In leaves, phenolic compounds concentrations were : chlorogenic acid > trans-ferulic acid > caffeic acid> vanillic acid, whereas in stem were : trans-ferulic acid > chlorogenic acid > vanillic acid and root contains only trans-ferulic acid. By providing 5 to 6 weeks gap between the sunflower harvest and sowing of next mungbean crop, will help in reducing the harmful allelopathic effects to provide the ambient growth conditions for the succeeding crops.

ACKNOWLEDGEMENT

We would like to thank Dr. Srikanta Prasad, Assistant professor, College of Horticulture, Arabhavi, University of Horticultural Sciences, Bagalakote for providing laboratory facility and chemicals analysis.

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