

Isolation and Identification of Allelochemicals of Sunflower (*Helianthus annuus* L.)

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ABSTRACT

Water extracts of stem and leaves of sunflower plant have been subjected to quantitative and qualitative analysis for the estimation and identification of the total phenols. It has been found that total phenols in the leaves were more (0.0316 mM/g) as compared to the stem (0.016 mM/g). The separation by thin layer chromatography has been found better than paper chromatography. Five allelochemicals (Chlorogenic, Caffeic, Syringic, Vanillic and Ferulic acid) in leaves, three in stem (Chlorogenic, ferulic and vanillic acids) and only one (ferulic acid) in the roots have been tentatively identified.

Key Words: Allelochemicals; Sunflower; Chromatographic separations

INTRODUCTION

Sunflower (*Helianthus annuus* L.) besides being the most important source of high quality vegetable oil also exhibit allelopathic effects on its subsequent crops and weeds (Leather, 1983). The allelopathic chemicals are released by the plants through volatilization, leaching or decomposition of their residues (Muller, 1966; Rice, 1984; Menges, 1988; Qasam & Hill, 1989). These substances have selective effects depending upon their concentrations, either inhibitory or stimulatory to the growth of companion or subsequent crops or weeds (Purvis *et al.*, 1985; Cheema, 1988). Sunflower is reported to be allelopathic in nature and its water extracts exhibited inhibitory effects both on radical and shoot growth of wheat as well as on subsequent crops and weeds (Wilson & Rice, 1968; Anderson *et al.*, 1978; Ghafar *et al.*, 2000).

The aim of the present study is to determine the amount of allelochemicals and to identify the chromatographically separated compounds exhibiting allelopathic effects in the sunflower extracts.

MATERIALS AND METHODS

Shoots and leaves of mature plants of HYSUN 33 sunflower variety were obtained from experimental fields of University of Agriculture, Faisalabad. They were dried, ground and stored in a desiccator containing anhydrous CaCl₂. Following the method of Bray and Thrope (1954), the total phenolics were estimated spectrophotometrically at 750 nm using chlorogenic acid as standard. The sunflower water extracts of roots, stems and leaves were subjected to paper and thin layer chromatographic separation procedures using various proportions of solvents. The identification of separated compounds was made by recording their R_f values and fluorescent colors emitted on exposure to UV lamp (Stahl, 1969; Qureshi *et al.*, 1975; Shaheen *et al.*, 1991).

RESULTS AND DISCUSSION

The amount of total phenolics in stem and leaves were 0.016 mM/g and 0.0316 mM/g, respectively. The amount has been found more in leaves as compared to stem as shown in Table I.

Table I. Total phenolic concentration of Sunflower leaves and stems

No.	Sample	Absorbance at 750nm	Total Phenols (mM/gm)
1	Stems	0.113	0.016*
2	Leaves	0.223	0.0316*

* Each value is a mean of 3 replicates

These findings support the previous work of various workers (Wilson & Rice, 1968), who explained that phenols being water soluble are widely distributed throughout the parts of plants and the amount of chlorogenic acid in comparison to other phenols has always been significantly higher (Brummette & Burns, 1972). They have also reported that phytotoxins also exert allelopathic effects in sunflower residues and which can be attributed to allelochemicals like chlorogenic, isochlorogenic acid and Scopolatin (Wilson & Rice 1968). Our findings are also confirmatory to the previous work as sunflower water extract inhibited the growth of wheat seedlings (Ghafar *et al.*, 2000).

Separation of Standard phenols and their mixtures have been tried on paper and thin layer chromatographic systems using five different solvent systems. It was concluded that thin layer chromatographic system using toluene: ethyle formate: formic acid (100:80:20) gave better resolution in comparison to the paper chromatographic system (Table II) as reported previously (Qureshi & Blain, 1974; Shaheen *et al.*, 1991).

Table II. R_f Values of standard phenols on TLC plate,

their colors under UV and after spray

No.	Std. Phenols	Rf. Values	Colours after Spray	Colours in UV light
1	Chlorogenic acid	0.05	Blue	Green
2	Syringic acid	0.57	"	Not visible
3	Vanillic acid	0.73	"	"
4	Ferulic acid	0.62	"	Blue
5	Caffeic acid	0.50	"	Light blue

Spraying reagent: a) 20% Na₂CO₃, b) Folin's reagent; Developing solvent system. Toluene: Ethylformate: Formic acid (100:80:20)

It is evident from Table III (for leaves) that in total, nine spots have been observed and five of them have been identified as chlorogenic, caffeic, syringic, ferulic and vanillic acids.

Table III. Allelochemicals identified from leaves of Sunflower plants

SETLCP ¹	Rf values	Colors in UV light	Colors after spray	Identified allelochemicals
1	0.051	Green	Blue	Chlorogenic acid
2	0.233	Not visible	"	Un-identified
3	0.4133	"	"	"
4	0.50	Light blue	"	Caffeic acid
5	0.57	Not visible	"	Syringic acid
6	0.62	Blue	"	Ferulic acid
7	0.73	Not visible	"	Vanillic acid
8	0.76	"	"	Un-identified
9	0.83	"	"	"

¹SETLCP= Spots eluted From TLC plate; Spraying reagent: a) 20% Na₂CO₃, b) Folin's reagent; Developing solvent system. Toluene: Ethylformate: Formic acid (100:80:20)

It can be observed (Table IV, for shoots) that seven spots have been separated and three of them have been identified as chlorogenic, ferulic and vanillic acids.

Table IV. Allelochemicals identified from shoots of Sunflower plants

SEFTLCP ¹	Rf values	Colors in UV light	Colors after spray	Identified allelochemicals
1	0.05	Light blue	Blue	Chlorogenic acid
2	0.53	Not visible	"	Un-identified
3	0.57	"	"	"
4	0.62	Blue	"	Ferulic acid
5	0.73	Not visible	"	Vanillic acid
6	0.77	Not visible	"	Un-identified
7	0.84	"	"	"

¹SETLCP= Spots eluted From TLC plate; Spraying reagent: a) 20% Na₂CO₃, b) Folin's reagent; Developing solvent system. Toluene: Ethylformate: Formic acid (100:80:20)

Table V indicates the separation of five spots on thin layer chromatographic plates recording Rf values, fluorescent color and after spray of carbonate and folin's reagent treatment but only ferulic acid has been identified from the sunflower root samples.

Table V. Allelochemicals identified from roots of Sunflower plants

SEFTLCP ¹	Rf values	Colors in UV light	Colors after spray	Identified allelochemicals
1	0.49	Not visible	Blue	Un-identified
2	0.62	Blue	"	Ferulic acid
3	0.66	Not visible	"	Un-identified
4	0.69	"	"	"
5	0.75	"	"	"
6	0.809	"	"	"

¹SETLCP= Spots eluted From TLC plate; Spraying reagent: a) 20% Na₂CO₃, b) Folin's reagent; Developing solvent system. Toluene: Ethylformate: Formic acid (100:80:20)

CONCLUSION

It is concluded that allelochemicals attributing allelopathic effects in the water extracts of sunflower plant contained chlorogenic, caffeic, vanillic, syringic and ferulic acids in leaves and chlorogenic, ferulic and vanillic acids in stem and only ferulic acid in roots of sunflower water extract.

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(Received 29 November 2000; Accepted 20 December 2000)