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Quantitative and Qualitative Determination of Allelochemicals In Sunflower (*Helianthus annuus* L.)

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Abstract: The amount of total phenolics quantitatively determined using spectrophotometric method in Sunflower Stem were 0.016 mM/g as compared to leaves (0.0316 mM/g). The tentatively identified phenols using thin layer chromatographic method on the bases of Rf-values and fluorescence colours in Sunflower shoot were chlorogenic, vanillic and ferulic acids. Chlorogenic, syringic, caffeic, ferulic and vanillic acids were also identified in the leaves but only ferulic acid was present in the root samples.

Key words: Allelochemicals, sunflower, quantitative and qualitative determinations

Introduction

Allelochemicals are natural chemical substances that can effect the growth of crops and weeds. These effects may be positive or negative depending upon the concentration and type of vegetations (Rice, 1994).

The nature of the allelochemicals produced as secondary substances in plants can be terpenoids and its derivative, cinnamic acid and derivative, coumarin, flavonoids, alkaloids and cyanohydrins (Ting, 1982). The allelopathic chemicals have been reported to play a role in plant-plant; plant-soil, plant-disease, plant-insect and plant-predators interaction that can be beneficial or detrimental to plants (Tang *et al.*, 1989). The interaction of woody perennial and agriculture crops when sown as mixed or inter or in rotation results in inhibition or stimulation of the growth of crop (Muller and Vegetatio, 1969). Sunflower (*Helianthus annuus* L.) is a high quality vegetable oil crop which is not strictly season bound and can be grown twice a year in Pakistan. Extracts of whole Sunflower plant significantly effects both radical and shoot growth of linseed (Mehboob *et al.*, 2000). The reported phytotoxins in sunflower residues are chlorogenic, iso chlorogenic, scopolin and suspected 1-naphthol derivative and they vary in their biosynthetic pathways, concentrations and localization to the species (Bansal and Bhan, 1993). The efficient utilization of Allelochemicals in cropping system will depend upon better understanding of chemicals involved and identification of these inhibitory or stimulatory chemical in any crop is a prerequisite to testing its allelopathic effects against crops. It is therefore, in this prospective that quantitative and qualitative estimation and identification of allelochemical present in the water soluble extract of Sunflower using spectro-photometric and chromatographic methods have been carried out.

Materials and Methods

Shoots and leaves of mature plants of SF-187 Sunflower variety were obtained in March 1999 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 chromatographic and thin layer chromatographic separation procedures using various proportions of solvents. The identification of separated compounds were made by recording their Rf values and fluorescent colours emitted on exposure to UV lamp (Shaheen *et al.*, 1991, Qureshi and Blain, 1974 Qureshi *et al.*, 1975).

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 1.

These findings support the previous work of various workers (Wilson and 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 and Burn, 1972). They have also reported that phytotoxins (Allelochemicals) also exert allelopathic effects in sunflower residues and these effects can be attributed to allelochemicals like chlorogenic, isochlorogenic acid and Scopolatin (Wilson and Rice, 1968). Our findings are also in confirmatory to the previous work as sunflower water extract effect the growth of linseed seedlings (Mehboob *et al.*, 2000).

Separation of Standard phenols and their mixtures have been tried on paper and Thin Layer Chromatographic Systems using seven different solvent systems. It was concluded that thin layer chromatographic system using toluene: ethyle formate: formic acid gave better resolution in comparison to the paper chromatographic system (Table 2). These studies are also in agreement to the earlier work of Shaheen *et al.* (1991), Qureshi and Blain (1974).

Rf Values, fluorescent colours under UV and after spray with 20% Na₂CO₃ and folin's reagent of separated spots of phenolic compounds isolated from sunflower leaves have been reported in Table 3. It can be seen that total nine spots have been observed and five of them have been identified as chlorogenic, caffeic, syringic, ferulic and vanillic acids.

Table 4 also shows the Rf values, fluorescence colourband

Table 1: Total Phenolic concentration of Sunflower leaves and stems

Sample	Absorbance at 750 nm	Total Phenols (mM/gm)
Stems	0.113	0.016*
Leaves	0.223	0.0316*

* Each value is a mean of 3 replicates.

Table 2: Rf value of standard phenols on TLC plate, their colour under UV and after spray

Std. Phenols	Rf. values	Colours after spray	Colours in UV light
Chlorogenic acid	0.055	Blue	Green
Syringic acid	0.57	"	Not visible
Vanillic acid	0.73	"	"
Ferulic acid	0.62	"	Blue
Caffeic acid	0.50	"	Light blue

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

Table 3: Rf values of leaves spots TLC, fluorescence colours and colours after spray of the spots of phenolic compounds isolated from Sunflower plants

Rf values	Colours in UV light	Colours after spray	Identified allelochemicals
0.051	Green	Blue	Chlorogenic acid
0.233	Not visible	"	Un-identified
0.4133	"	"	"
0.50	Light blue	"	Caffeic acid
0.57	Not visible	"	Syringic acid
0.62	Blue	"	Ferulic acid
0.73	Not visible	"	Vanillic acid
0.76	"	"	Un-identified
0.83	"	"	"

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

Table 4: Rf values of Shoot spots TLC, fluorescence colours and colours after spray of the spots of phenolic compounds isolated from Sunflower plants

spots eluted	Rf value from TLC plate	Colour in UV light	Colours after Spray	Identified allelochemicals
1	0.05	Light blue	Blue	Chlorogenic acid
2	0.53	Not visible	"	Un-identified
3	0.57	Not visible	"	Un-identified
4	0.62	Blue	"	Ferulic acid
5	0.73	Not visible	"	Vanillic acid
6	0.77	Not visible	"	Un-identified
7	0.84	Not visible	"	Un-identified

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

Table 5: Rf values Root spots TLC, fluorescence colours and colours after spray of the spots of phenolic compounds isolated from Sunflower plants

spots eluted	Rf value from TLC plate	Colour in UV light	Colours 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	"	"	"

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

after spray of the spots of phenolic compounds isolated from sunflower stem. It can be observed that seven spots have been separated and three of them have been identified as chlorogenic, ferulic and vanillic acids.

Table 5 indicates the separation of five spots on thin layer chromatographic plates recording Rf values, fluorescence colour and after spray of carbonate and folin's reagent treatment but only ferulic acid has been identified from sunflower root samples.

It is concluded that inhibitory/stimulatory substances 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.

References

- Bansal, G.L. and V.M. Bhan, 1993. Status of research on allelopathy and future scope of work in India. *Indo J.A. Sci.*, 63: 769-776.
- Bray, H.G. and W.V. Thrope, 1954. In *Methods in Biochemical Analysis* (David Glich ed) 1: 27.
- Brummette, B.T. and E.E. Burn, 1972. *Plant Phenolics*. *Food Sci.*, 37: 1.
- Cheema, Z.A., 1988. Weed control in wheat through sorghum Allelochemicals. Ph.D. Thesis, Univ. Agriculture Faisalabad, Pakistan.
- Mehboob, N., Bushra Saleem and M. Jamil Qureshi, 2000. Allelopathic effects of Sunflower (*Helianthus annuus*). *Pak. J. Biol. Sci.*, 3: 1305-1307.
- Muller, C. and H. Vegetatio, 1996. As reported by George R. Waller in ACS Symp. Schicago, Illinois Sept. 8-11, 1985. *Amer. Chem. Soc. Washington D.C.*, 18: 257.
- McWorther, C. G., 1984. Future needs in weed science. *Weed Sci.*, pp. 179-81.
- Qureshi, M.J. and J.A. Blain, 1974. Isolation and identification of antioxidant factors in tomato. *Nucleus*, 11: 25-32.
- Qureshi, M.J., M.H. Naqvi and M.Mohyuddin, 1995. Isolation and identification of polyphenoloxidase substrate in "Liquot" (*Eriobotrya japonica*). *Pak. J. Chemistry*, 8: 9-12.
- Rice, E.L., 1994. "Allelopathy" Second Edition, Orlando, Florida, Academic Press, USA.
- Shaheen, T., Riaz Ahmad and M. Jamil Qureshi, 1995. Total phenolics of Neem Plant. *Pak. J. Sci.*, 33: 1-10.
- Tang, C.S., K.Komai and R.S. Huang, 1989. *phytochemical ecology: allelochemicals, mycotoxins, insect pheromones and allomones*, Chon, C.H. and George R. Waller, Eds. Institute of Botany, Academia Sinica Monograph Series 9, Taipei, Roc., pp: 217-226.
- Ting, I.P., 1982. "Natural Products, in Plant Physiology" Addison Wesley Pub. Co. Inc., USA., pp: 302-325.
- Wilson, R.E. and E.L. Rice, 1968. Allelopathy as expressed by *Helianthus annuus* and its role in old field succession. *Bull. Torrey Bot. Club*, 95: 432-448.