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*Short Communication*

# Quantification of alkaloids, phenols and flavonoids in sunflower (*Helianthus annuus* L.)

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**Allelochemicals in leaves, stems and roots of sunflower (cv Hysun 38) were determined using thin layer chromatography (TLC) for alkaloids and spectrophotometry for phenols and flavonoids. In the TLC, the highest $R_f$ value was recorded in leaves, followed by roots and stems, a sequence that held true also for the quantity of phenols and flavonoids. The soil texture, which was sandy loam, was not changed before or after the harvest.**

**Key words:** Allelochemicals, alkaloids, flavonoids, phenols, sunflower, spectrophotometry, thin layer chromatography.

## INTRODUCTION

Allelochemicals in plants are mostly secondary metabolites, or by-products of primary metabolic processes (Levin, 1976) that exert an allelopathic effect on the growth and development of the plant in which they are produced or on neighboring plants.

Secondary compounds are metabolically active in plants and microorganisms, and the biosynthesis and biodegradation of these chemicals play important roles in the ecology and physiology of organisms that produce them (Waller and Nowacki, 1978; Waller and Dermer, 1981). Some of them are accumulated at various stages of growth, and the extent of accumulation of some compounds depends on the time of the day and on the season. Allelochemicals in plants have been documented by several workers. The chemicals are produced in plant organs that are either above or below the ground, or both, and cause allelopathic effects in a wide range of plant communities. Different plant parts contain varying amounts of allelochemicals (Rice, 1974): roots contain smaller amounts than leaves but in some plants, it is the reverse; sometimes stems are the principle source of toxicity and often leaves are the most important sources of allelochemicals. Specific inhibitors in leaves have been reported by many workers. A major prerequisite for allelopathy is the transport of allelochemicals from one plant part to another. Therefore, the mode of transfer may play a great role in toxicity and persistence of allelochemicals. The plants generally store these chemicals in cells in the bound forms, for example as water-soluble glycosides, alkaloids, flavonoids and phenols; the chemicals are therefore not toxic to the donor plant. However, once released into the environment, allelochemicals may be either degraded or transformed into other forms and these forms may affect the neighboring receiver plants and may even prove toxic to the plant that produced them (autotoxicity). Such release usually follows their breakdown by plant enzymes. Allelochemicals are released into the environment from special glands on the stem and leaves. Therefore, it is necessary to examine the relative abundance of allelochemicals in leaves, roots and stems.

## MATERIALS AND METHODS

Sunflower plants (cv Hysun 38) were grown in pots in the Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. Three seeds were sown in each pot and these were given a basal dose of 2 g of diammonium phosphate and 1 g each of urea and potash. When the plants had reached the vegetative stage (40 days after sowing), they were uprooted and separated into their constituent parts, namely: leaves, stems and roots. All the parts were washed thoroughly with distilled water, dried, pulverized in a mill and stored until further use in a cool place (5°C) along with anhydrous $CaCl_2$ to keep them dry.

**Soil analysis**

***Particle-size distribution (percentage of sand, silt and clay)***

50 g of soil along with 50 ml of a dispersing reagent (2% sodium hexametaphosphate) were transferred to a stirring cup and left overnight. The suspension was then stirred for 15 min and transferred to a 1000 ml graduated cylinder. Distilled water was added to the graduated cylinder to make up the volume to 1 L. The suspension was stirred vigorously with a metal plunger. The first hydrometer reading (R1) and the temperature were recorded after 2 h. The percentage of sand, silt and clay was calculated after adjusting the values to the temperature. All the readings were taken as described by Brady (1990).

The textural class of the soil was determined by using the textural triangle (US Department of Agriculture classification System.

% Separate (sand, silt or clay) = CHR/wt of soil taken × 100

Where, CHR is the corrected hydrometer reading (after adjusting for temperature).

**Total phenols**

Total phenols were determined by using the Folin Ciocalteu reagent (McDonald et al., 2001). A dilute extract of each plant extract (0.5 ml of 1:10 g/l) or gallic acid (the phenolic compound commonly used as the standard) was mixed with the Folin Ciocalteu reagent (5 ml of the reagent diluted tenfold with distilled water) and aqueous $Na_2CO_3$ (4 ml, 1 M). The mixtures (each with a separate extract representing leaves, stems or roots) were allowed to stand for 15 min and total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200 and 250 $mgL^{-1}$ solutions of gallic acid in methanol/water (50:50 v/v). The total phenol value was expressed in terms of gallic acid equivalent (mg $g^{-1}$ of dry mass), which is a commonly used reference value.

**Total flavonoids**

Aluminum chloride colorimetry was used for flavonoids determination (Chang et al., 2002). Each plant extracts (0.5 ml of 1:10 g/l) of each organ in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 M potassium acetate and 2.8 ml of distilled water. After keeping the mixture at room temperature for 30 min, the absorbance of the reaction maximum was measured at 415 nm with a double beam UV/visible spectrophotometer (Perkin Elmer, USA). The calibration curve was prepared by using quercetin solutions at concentrations from 12.5 to 100 µg of quercetin per ml of methanol.

**Alkaloids**

Alkaloids were extracted from 2 g samples of the dried plant organs (air-dried leaves, stems and roots) with n-hexane (3 × 20 ml) followed by MeOH (3 × 20 ml). The extraction was done at room temperature and each cycle with hexane or MeOH lasted for 24 h. The extract was evaporated in a rotary film evaporator (RFE). As the last step, each tube was rinsed with 15 ml of distilled water, which was added to the flask. The pH was adjusted to 2.0 with 5% $H_2SO_4$ and the contents were extracted with $CH_2Cl_2$ (3 × 1/3 of the total volume) to separate the non-alkaloids in the mixture. The acidic aqueous solution was made alkaline (pH 8 to 10) by adding 10% NaOH and the contents were extracted with $CH_2C1_2$ to obtain the alkaloids (Ulubelen, 2000). At this stage, the aqueous phase was discarded and the organic phase was dried in RFE. The residue was dissolved in 1 ml $CH_2Cl_2$ and stored until further use.

**Thin layer chromatography (TLC)**

***Preparation of TLC plates***

The glass plate, which measured 20 × 20 cm, was prepared for TLC by coating it to a thickness of 0.25 mm with silica gel HF254 (Article no. 7739, Merk) using a TLC spreader.

***Spotting the plates***

The samples were spotted on the TLC plates with microcapillary tubes and the prepared (spotted) TLC plates were eluted using a solvent system comprising toluene, ethyl acetate and diethylamine (6:2:0.5) (Ulubelen, 2000).

**Confirmatory test for alkaloids**

Immediately after visualization under UV light, the plate was sprayed with the Dragendorff spray reagent to confirm the spots for the positive test.

**Visualization of the TLC plates**

The plates were examined under UV at 254 and 365 nm. The spots were marked with a pencil. The value for each compound as evident from the blue and green florescent spots under UV was calculated as the Rf value (retention factor) for that compound:

Rf value = Distance traveled by the compound / Distance traveled by the solvent front

**Statistical analysis**

The statistical significance of the differences in the quantities of allelochemicals from the different plant parts (leaves, stems and roots) was evaluated with a Mann-Whiney U test. P values less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

### Alkaloids

The methanol extracts of the sunflower leaves, stems and roots for over two years showed different banding patterns following TLC and also showed different Rf values under UV light at 365 nm (Table 1). All bands from the leaves showed less polarity than those from the roots and stems. The number of bands was also the highest in the leaves. All these results may be due to the fact that volatilization of the alkaloids was minimal because of the low temperatures in Islamabad. Secondly, alkaloids from roots are lost through leaching and other micro-environmental factors (Asa and Karlsson, 1998). Differences in chemical composition and diversity may also account for the differences among the plant parts in terms of their alkaloid contents (Petaraitis et al., 1989;

**Table 1.** R*f* values of sunflower (cv Hysun 38) determined by thin layer chromatography.

| Plant part | $R_f$ values (cm) | |
|---|---|---|
| | 1st year | 2nd year |
| Leaves | 0.98000 ± 0.00577 | 0.90000 ± 0.0289 |
| Stems | 0.7400 ± 0.0153 | 0.69000 ± 0.00577 |
| Roots | 0.8867 ± 0.0418 | 0.8300 ± 0.0252 |

Each value is the average of three experiments ± standard deviation.

**Table 2.** Flavonoids and phenol contents of sunflower (cv Hysun 38) determined by spectrophotometry.

| Plant part | Phenol (mg/g) | | Flavonoid (mg/g) | |
|---|---|---|---|---|
| | 1st year | 2nd year | 1st year | 2nd year |
| Leaves | 311.67 ± 4.41 | 400.00 ± 5.77 | 83.33 ± 3.76 | 100 ± 3.61 |
| Stems | 200.00 ± 5.77 | 256.7 ± 12.0 | 45.00 ± 2.89 | 65.67 ± 2.69 |
| Roots | 270.00 ± 5.77 | 342.3 ± 24.3 | 70.00 ± 5.77 | 85.00 ± 2.89 |

Each value is the average of three experiments ± standard deviations.

Roesenzweig and Abransky, 1993; Olanbanji et al., 1997; Asa and Karlsson, 1998).

## Phenols

It is shown in Table 2 that the amount of the total phenols was maximum in the leaves, followed by roots and stems. These results are in agreement with those obtained by Rice (1974), who also reported that more allelochemicals were produced in the second year because of higher temperatures that prevailed during that year.

## Flavonoids

The data on flavonoids (Table 2) show that the maximum amount of flavonoids was produced in the leaves followed by roots and stems. The order remained the same in the second year. It is likely that leaves contain the most allelochemicals because those in roots are lost by leaching and those from stems are translocated. The chemicals are also not volatilized from the leaves.

## Conclusions

In sunflower, the content of allelochemicals was highest in the leaves, followed by the roots and stems, respect-tively. Stress stimulates the production of allelochemicals.

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