

## Allelopathic potential of *Cyperus rotundus* L. II. Isolation and identification of phytotoxins

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### ABSTRACT

In earlier work we had found that Purple nutsedge (*Cyperus rotundus* L.) has a strong allelopathic interference with several test crops. In this study Chromatographic analyses using HPLC have revealed the presence of ferulic, caffeic, hydroxyl benzoic, syringic, chlorogenic, p-coumaric acids and 3 unidentified compounds in *C. rotundus* shoot and the same phenolic acids and one unidentified compound in the tuber. Additional chromatographic analyses showed that the shoot and tuber of *C. rotundus* also contained 13 and 11 volatile compounds, respectively. Of these 10 identified compounds were :  $\alpha$ -pinene,  $\beta$ -pinene, limonene, camphor, camphene, citronellal, farnesol and petalostemumol. These isolated compounds have been reported to be phytotoxic against several plant species.

**Key words:** Allelopathy, phenolic acids, phytotoxins, purple nutsedge, shoot, tuber, volatile compounds.

### INTRODUCTION

Allelopathy is an important mechanism of plant interference mediated through addition of plant produced allelochemicals to the rhizosphere (7,11). The ecological role of allelopathy in ecological and manipulated ecosystems has attracted the attention of many scientists. Rice (13) summarized the role of allelopathy in plant succession, structure of plant community, vegetation patterning and plant productivity. Allelochemicals are released from the allelopathic plants through volatilization, leaching by precipitation (rain, dew, mist, fog, snow) and root exudation and decomposition of plant residues in soil (13). Many reports indicated that the inhibitory substances involved in allelopathy are phenolic compounds and terpenoids (5,9,15). In an earlier work (1), we found that allelopathy is the main component of interference of purple nutsedge with the test crops. Volatile and non volatile inhibitory compounds were from *C. rotundus* released through root exudates, volatilization and decaying residues. However, the nature of inhibitory compounds was not investigated. Therefore, this study was aimed to identify the chemical compounds responsible for the allelopathic effects of purple nutsedge.

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## MATERIALS AND METHODS

The procedure used for extraction of phenolic compounds was basically that of Harborne (8). Five grams of air-dry powder of tops and tubers were soaked separately in 50 ml of 70% ethanol for 48 h under laboratory condition and filtered through Whatmann # 1 filter paper. The filtrate was concentrated to 5 ml at 70°C using flash evaporator and re-extracted with equal volume of petroleum ether to remove the lipid and other non-polar compounds using separating funnel. The remaining extract which is mostly water extract was further concentrated to 2 ml and used for chromatographic analyses.

### Separation and identification of Phenolic compounds

Two micro liters of extract were injected in a Shimatzu – C-6A high pressure liquid chromatograph. The conditions of separation and identification are outlined in Table 1. Two micro liters from standard phenolic compounds purchased from Fluka Company were injected under the same experimental conditions and used for identification and quantification of the compounds in the sample.

Table 1. Conditions of high pressure liquid chromatograph (HPLC) used to separate the phenolic compounds

Parameter	Value
Initial temperature t <sub>1</sub>	57 °C
Final temperature t <sub>2</sub>	200 °C
Temperature rate	4 C / minutes
Injection temperature	210 °C
Detector temperature	210 °C
Flow rate of the carrier gas	25 cm / second
Column dimensions	30 m length × 0.25 mm diameter
Diatomite	Supleco wax 10
Attenuation	0.01 ppm
Rate of recorder	10 mm / minutes
Detector	Flame ionization detector (FID)
Size of injection sample	1 micro liter

### Separation and identification of volatile compounds

The procedure used for separation and identification of volatile compounds was of Alsaadawi *et al.* (3). Ten grams fresh weight of tops and tubers were chopped in to pieces and placed separately inside conical flasks of 250 cm<sup>3</sup> size. The flasks were sealed with rubber stopper and placed for one hour under laboratory condition For identification, one micro liter from the atmosphere in side each flask was taken by a syringe and injected inside a Shimatzu gas chromatogram equipped with 30 m × 0.25 mm i. d. fused silican column packed with Supleco wax 10. The temperature programming was set from 57 to 200°C at increasing rate of 4 °C / min. The flow rate of gases was: N, 25 ml / sec.; Helium 25 ml / sec.; air 200 ml / sec. The oven temperature was 210 C and injection temperature was 210°C. The chart speed was 10 mm / minute. The attenuation time was set at 0.01 ppm. One micro liter from standard volatile compounds purchased from Fluka Company was injected under the same experimental conditions and used for identification and quantification of the compounds in the sample.

## RESULTS

Chromatographic analyses using HPLC revealed the presence of ferulic, caffeic, hydroxyl benzoic, syringic, chlorogenic, p-coumaric acids and three unidentified compounds in the shoot and the same phenolic acids and one unidentified compound in the tuber of purple nutsedge plant (Figs. 1 and 2). Quantitative analyses indicated that ferulic and caffeic acids were found to be higher in top than in tubers while concentration of chlorogenic acids is higher in tubers than in top of purple nutsedge (Table 2). The other phenolic acids did not show significant differences in their concentrations in top and tuber. Total phenolic acids appeared to be slightly higher in top than in tuber.

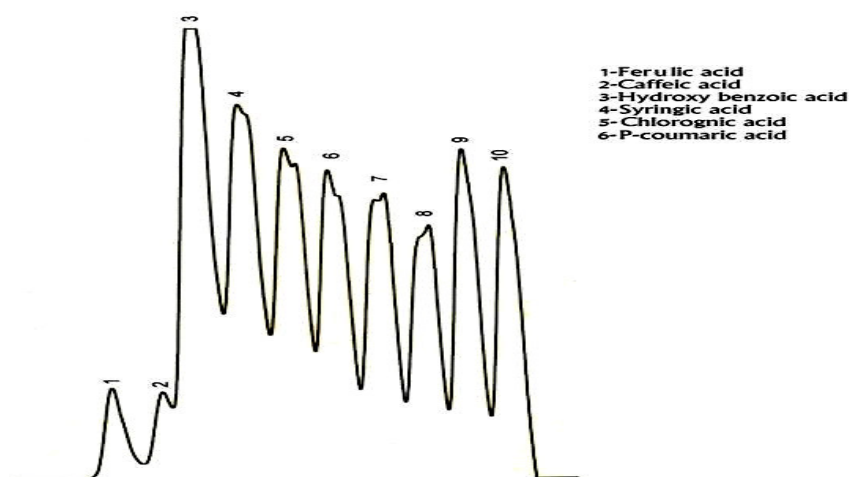


Figure 1. HPLC of phenolic acids in shoot extract of purple nutsedge.

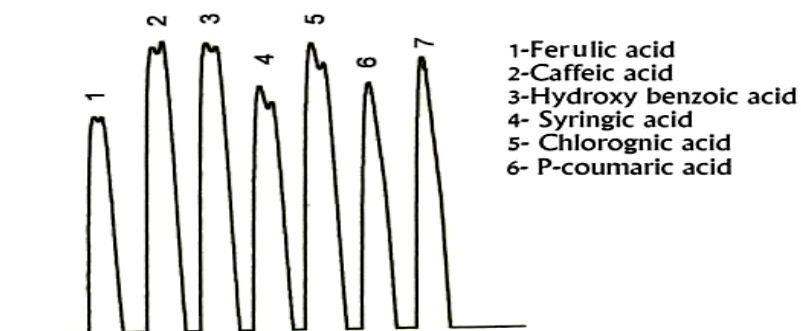


Figure 2. HPLC of phenolic acids in tubers extract of purple nutsedge.

Table 2. Concentration of phenolic acids present in shoot and tuber extracts of purple nut sedge

Phenolic acids	Attenuation time (minutes)	Concentration ( $\mu\text{g} / \text{ml}$ )	
		shoot	Tubers
Ferulic acid	4.2	41.247*	31.959
Caffeic acid	4.9	36.519*	16.457
Hydroxyl- benzoic acid	5.6	61.575	61.279
Syringic acid	6.3	29.887	29.802
Chlorogenic acid	7.0	15.674	35.552*
p- Coumaric acid	7.7	27.121	27.428
Total	----	212.023	202.477

\* Significantly different from each other at 0.05 according to Student's t test.

Chromatographic analyses showed the presence of 13 and 11 volatile compounds in top and tuber respectively (Figs. 3 and 4). Ten of these compounds were identified as  $\alpha$ -pinene,  $\beta$ -pinene, limonene, camsaicin, artemisin, camphene, camphor, citronellal, farnesol and petalostemumol. Concentrations of all identified compounds except Camphene, Camsaicin and Limonene were found to be higher in tuber than in top (Table 3). Moreover total volatile compounds appeared to be considerably higher in tuber than in top.

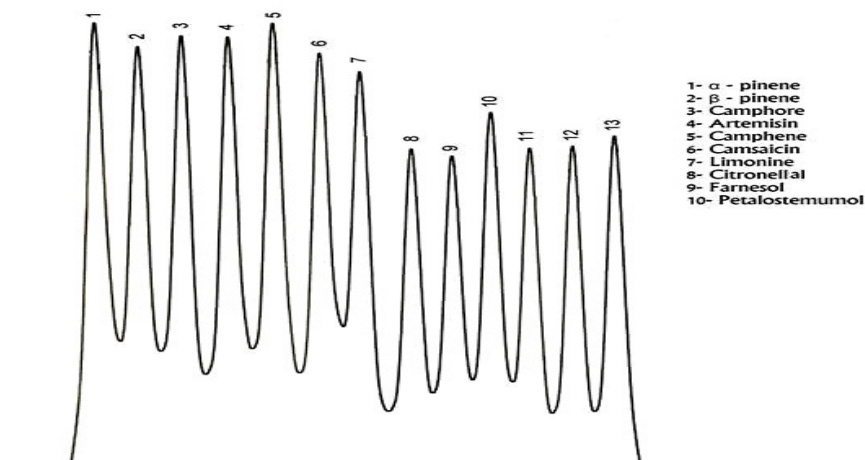


Figure 3. Gas chromatogram of atmosphere containing macerated shoot of purple nutsedge.

## DISCUSSION

In a previous work (1), we reported that purple sedge exerted a strong allelopathic potential against several test crops. The inhibitory compounds appeared to be released through root exudation, volatilization and decaying of plant residues. The present study clearly revealed that purple sedge released volatile and non volatile compounds into the

soil and inhibited the growth of test species. However the degree of phytotoxicity was dependent on the receptor plant species and this could explain the variation in the response of growth of the test species to allelopathic effects of purple nutsedge.

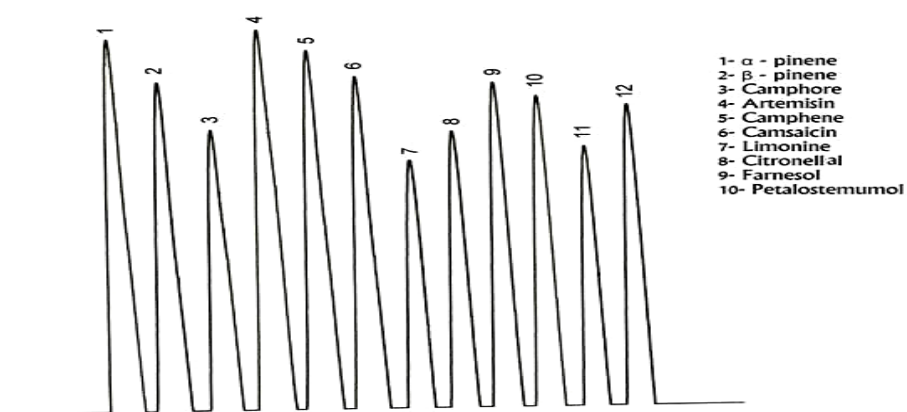


Figure 4. Gas chromatogram of atmosphere containing macerated tubers of purple nutsedge.

Table 3. Concentration of volatile compounds present in atmosphere of macerated shoot and tuber of purple nutsedge

Volatile compounds	Attenuation time (minutes)	Concentration ( $\mu\text{g} / \text{ml}$ )	
		Shoot	Tubers
A – pinene	4.4	56.071	61.957
B – pinene	5.2	56.141	65.019
Camphor	5.9	66.806	66.665
Artemisin	6.7	54.310	66.921
Camphene	7.4	59.925	64.899
Camsaicin	8.2	57.466	62.341
Limonene	8.9	60.910	56.287
Citronellal	9.6	56.449	66.937
Farnesol	10.3	51.838	60.009
Petalostemumol	10.9	52.289	69.100
Total	---	572.205	640.135

All non volatile compounds identified were phenolic acids. Phenolic compounds are known to exude from plant roots or be produced during decay of plant residues (2,4,6,9,14). All the identified phenolic acids are reported to affect the seed germination and seedling growth of the plant species. Rice (13) pointed out that a large number of phenolics are allelopathic agents that play a significant role in several ecological phenomena such as seed dormancy, plant succession, vegetational patterning (5, 15, 9).

Our results also exhibited the presence of volatile compounds in top and tuber of purple nutsedge. All the identified non-volatile compounds were reported to have strong

allelopathic potential against plants with the exception of artemisin and camsaicin. Muller (12) indicated that terpenes released from some *Salvia* inhibited the seedling growth by reducing cell division and cell elongation of cucumber seedling. Kohli *et al.*, (10) mentioned that volatile oil from *Eucalyptus* spp reduced chlorophyll content and respiratory activity of *Parthenium hysterophorus*. Alsaadawi *et al.*, (3) revealed that volatile compounds isolated from senescent and non-senescent sour orange *Citrus aurantium* leaves were inhibitory to seed germination and seedling growth of *Amaranthus retroflexus*. No attempt was done to determine the allelopathic potential of artemisin, Petalostemum and camsaicin against germination and growth of plant species.

The higher amount of volatile compounds in tubers compared to shoot could explain the high toxicity of macerate tuber on the test specie compared to macerated shoot tissue, since no significant differences were observed between shoot and tubers extracts of purple nutsedge in a particular phenolic compound and in the total phenolics.

The evidence is thus strong that purple nutsedge contain volatile and non-volatile inhibitors that reduce germination and seedling growth of test species. This allelopathic potential is considered as an important component of the interference exerted by purple nutsedge against the test crops.

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