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HESPERETIN 7-RUTINOSIDE (HESPERIDIN) AND TAXIFOLIN 3-ARABINOSIDE AS GERMINATION AND GROWTH INHIBITORS IN SOILS ASSOCIATED WITH THE WEED, *Pluchea lanceolata* (DC) C.B. CLARKE (ASTERACEAE)

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Abstract—Hesperetin 7-rutinoside (Hesperidin) and taxifolin 3-arabinoside were detected in the soils associated with the rapidly spreading perennial weed, *Pluchea lanceolata*. In the present investigations, inhibitory potential of the aqueous extracts of the two compounds was established and confirmed through growth experiments pertaining to seed germination and seedling growth of radish, mustard, and tomato, with 10^{-4} M solutions of the authentic samples. The significance of the water-soluble compounds present in the rhizosphere zones of the weed and its interference potential is commented upon.

Key Words—Allelopathy, growth experiments, hesperidin, interference, *Pluchea lanceolata*, taxifolin 3-arabinoside.

INTRODUCTION

Previously, Inderjit and Dakshini (1990) reported that *Pluchea lanceolata* (DC) C.B. Clarke (Asteraceae) achieves its interference potential by releasing and inhibiting seed germination and seedling growth of different plant species through phytochemicals synthesized by the weed. These studies necessitated the identification of phytochemical compounds in the soils associated with the weed. Since a large number of phenolic compounds have been shown to possess

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allelopathic potential (Rice, 1984), isolation and characterization of only this category of compounds have been undertaken. This analysis resulted in the detection of the flavonone, hesperetin 7-rutinoside (hesperidin), and a dihydroflavonol, taxifolin 3-arabinoside, from the soils associated with the weed. Since the occurrence of these compounds in the soil has not been reported earlier, investigations on their effects on germination and growth of some plant species have been undertaken and data pertaining to these are reported here.

METHODS AND MATERIALS

The soils associated with and without (as controls) the weed, *Pluchea lanceolata*, were collected from the fields around the metropolitan city of Delhi. These samples were air-dried and stored in paper bags, to be used later for extraction of compounds.

Extraction, Purification, and Identification of Phenolic Constituents. The soil extracts (5 : 1, v/w) were prepared by shaking the soil samples with double distilled water (DDW) for 1 hr at room temperature. The extracts were filtered, dried in vacuum, and the residue extracted with 10 ml of methanol. These extracts were loaded on Whatman No. 3 (46 × 57 cm) chromatographic paper and developed by descending chromatography using the butanol–acetic acid–water (BAW, 4 : 1 : 5, upper phase) (Harborne, 1973). Chromatograms were dried and scanned bands were marked under UV ($-NH_3$) and UV($+NH_3$). Six bands appeared on the chromatogram with extracts from weed-associated soil, whereas only two bands were detected on the chromatogram with control soil extracts. Each band was eluted in methanol, loaded again separately, washed repeatedly with DDW, and finally extracted with methanol. To confirm the purity of these compounds, each compound was loaded on Whatman No. 1 chromatographic paper and run in four solvent systems: BAW, DDW, 15% acetic acid, and forestal (conc. HCl–acetic acid–water, 3 : 30 : 10). After confirming the purity of these compounds, each was again loaded on Whatman No. 3 chromatographic paper and washed repeatedly with DDW through descending chromatography. The compounds from chromatograms were finally eluted with methanol, and the eluates then were taken for spectral analysis and shifts (NaOH, $AlCl_3$, $AlCl_3$/HCl, NaOAc, and NaOAc/H_3BO_3), if any, of the parent as well as the hydrolyzed fractions. These purified compounds, after loading separately on Whatman No. 1 chromatographic paper, were developed through descending chromatography and the fluorescence (UV and UV+NH_3) and R_f (×100) values were recorded in the four solvent systems mentioned above. The DDW residue of the hydrolyzed parent compound was used for the characterization of sugar moiety, if any, following Harborne (1973). Further, the glycoside and aglycone forms were cochromatographed with the authentic samples and compared with data given by Mabry et al. (1970). The two compounds

common to both (control and weed-associated soil) were not considered for further study. Of the remaining four, two were phenolic acids and the others a flavonone and a dihydroflavonol. Since the former class of compounds has been reported earlier (Rice, 1984) in many allelopathic studies, only the last two compounds were subjected to further analysis.

Seed Germination and Growth Experiments. To investigate the allelopathic potential of the identified compounds, germination and growth experiments were performed. Since the synthetic glycoside of taxifolin (taxifolin 3-arabinoside) was not available, the synthetic aglycone (taxifolin) was used for germination studies. It could be argued that with such a step the comparison of results of two compounds may not be fully justifiable. However, in view of the observations of Rice and Pancholy (1974) that sugars are easily split from the glycoside fraction by microbial action and they tested aglycones for inhibitory activity, the results obtained in the present investigations can be compared.

The compounds hesperidin and taxifolin were dissolved initially in methanol, dried in vacuum, and dissolved in DDW to a final concentration of 10^{-4} M. In general, this low concentration of phytotoxins has been shown to be inhibitory to germination and seedling growth (McCahon et al., 1973; Williams and Hoagland, 1982). The seeds of commonly grown crops in the region, e.g., radish (*Raphanus sativus* var. pusa desi), mustard (*Brassica juncea* cv. PR 45), and tomato (*Lycopersicon esculentum* var. pusa ruby) were selected to study the allelopathic potential of the compounds. For germination studies, 50 seeds of these plants were sown on filter paper moistened with DDW (served as control) or compounds, each in equal volume, and placed in 15-cm-diam. Petri plates. To maintain uniform moisture status in the Petri plates, a cotton pad soaked in DDW or compounds was placed below the filter paper. Root and shoot lengths were recorded every 24 hr for seven days. Each treatment was replicated three times. During the period of experimentation, the temperature regime of 30 $\pm$ 5°C and diurnal regime of light condition were maintained. After seven days, fresh weight of three sets of five seedlings each was recorded. To get a better evaluation of the inhibitory effect, relative germination (RG) index was calculated as follows:

$$\text{RG index} = \frac{\text{number of seeds germinated at 48 hr} \times 100}{\text{number of seeds germinated at 168 hr}}$$

Comparison of the various parameters was made using one way analysis of variance.

RESULTS AND DISCUSSION

A flavonone, hesperidin, and a dihydroflavonol, taxifolin 3-arabinoside, were identified in the aqueous extracts of the soils associated with the weed, *Pluchea lanceolata* (Tables 1 and 2). Compared to taxifolin, hesperidin was a

TABLE 1. FLUORESCENCE, R_f (×100) VALUES, AND ABSORPTION SPECTRA OF COMPOUNDS ISOLATED FROM SOILS ASSOCIATED WITH *Pluchea lanceolata*

	Fluorescence[a]		*Rf* (×100) values[b]				Absorption spectra						
Compound	UV	UV + NH_3	DDW	BAW	HOAc	For-estal	Methanol	NaOH	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3	Identification
1	Y	GY	88	49	70	83	272, 326	286, 379	272, 322sh 387	271, 322sh 374	272, 306sh 329	272, 306sh 325	Hesperidin
2	BM	BM	87	48	84	78	279, 312sh[c]	285, 353	278, 306	278 304sh	276, 350	278, 350	Taxifolin 3-arabinoside

[a] Fluorescence key: Y, yellow; GY, green yellow; BM, brown mauve.
[b] Solvent key: DDW, double distilled water; BAW, butanol–acetic acid–water (4 : 1 : 5, upper phase); HOAc, 15% acetic acid; forestal, conc. HCl–acetic acid–water (3 : 30 : 10).
[c] sh-shoulder.

TABLE 2. PAPER CHROMATOGRAPHY OF SUGAR IN WATER FRACTIONS AFTER ACID HYDROLYSIS OF TAXIFOLIN GLYCOSIDE

Compound	R_f (×100) values[a]		Color with aniline hydrogen phthalate[b]
	BAW	BBPW	
Suspected Arabinose	16.5	25.5	Pink red
Known Arabinose	17.0	25.7	Pink red

[a] Solvent key: BAW, butanol–acetic acid–water (4 : 1 : 5, upper phase); BBPW, butanol–benzene–pyridine–water (5 : 1 : 3 : 3).
[b] Prepared by dissolving 9.2 ml aniline and 16 g phthalic acid in 490 ml of butanol, 490 ml diethyl ether, and 20 ml water.

stronger inhibitor of the seed germination of the three crop species (Table 3). Hesperidin also inhibited significantly ($P < 0.05$) the growth of roots and shoots of all seedlings. Similarly, taxifolin brought about reduction in roots and shoots of the seedlings ($P < 0.05$), excepting shoot growth of mustard and root growth of tomato (Table 3). However, on a relative basis, hesperidin inhibited the shoot growth more; taxifolin, on the other hand, suppressed the root growth more effectively except in tomato, where hesperidin was more inhibitory. Similarly, both compounds reduced the fresh weight of the seedlings of all species ($0.01 < P < 0.08$) except mustard seedlings grown with hesperidin. Like shoot growth, fresh weight of seedlings was affected more by hesperidin than taxifolin. Furthermore, tomato seeds were found to be more sensitive to the compounds than the other two crop species (except grown with taxifolin). It may be noted that the effect of these two compounds varied with seeds tested. Even though the reason for this variation can not be explained, it is likely that, as opined by Williams and Hoagland (1982), this may be due to differences in seed size, seed coat permeability, differential uptake, and metabolism. However, any inference in this regard requires further investigation.

Additionally, seedlings grown with either of the compounds showed browning of the root tip and root–shoot (hypocotyl) zones. Whether this resulted from increased reduction of ascorbic acid in the xylem vessels, as thought by Rice (1984), or from simple oxidation of phenolic compounds needs further study. In spite of earlier suggestions regarding the probable effects of flavanoids on different physiological parameters (Koeppe and Miller, 1974; Lang and Racker, 1974), mechanisms leading to suppression of seedling growth are not clear. However, the present study has shown that two compounds are potent allelochemics associated with the rhizosphere in soils of the weed. Furthermore, their role in interference of seed germination and seedling growth of crop spe-

TABLE 3. EFFECT OF COMPOUNDS (10^{-4} M) ON SEED GERMINATION AND SEEDLING CHARACTERISTICS OF DIFFERENT PLANT SPECIES

Plant species	Treatment[a]	Germination (%)	RG index[b]	Root length (cm) days after sowing			Shoot length (cm) days after sowing			Fresh weight (g)[c]
				1	4	7	1	4	7	
Mustard	C	92	100	0.34 ±0.42	4.89 ±1.94	5.22 ±1.74	0.14 ±0.04	1.96 ±0.80	2.54 ±0.76	0.1726 ±0.019
	H	66	66.60		2.20***[d] ±1.20	4.16* ±2.14		0.61*** ±0.42	1.56*** ±0.97	0.1479 ±0.021
	T	98	97.95	0.28 ±0.11	3.47** ±2.04	4.10* ±2.41	0.12* ±0.06	1.53* ±0.81	2.16 ±1.31	0.1520* ±0.0070
Radish	C	100	100	0.43 ±0.20	2.82 ±1.58	3.95 ±1.40	0.30 ±0.13	1.58 ±0.82	3.01 ±1.58	0.3484 ±0.046
	H	80	100	0.29** ±0.41	1.85*** ±0.87	2.68*** ±1.29		0.80*** ±0.37	1.58*** ±0.91	0.2651* ±0.005
	T	98	100	0.27* ±0.14	1.71*** ±0.82	2.14*** ±1.07	0.23 ±0.16	1.37** ±0.61	1.98*** ±0.80	0.2910* ±0.004
Tomato	C	54	7.40		1.74 ±1.24	3.95 ±1.49		0.72 ±0.04	2.67 ±1.49	0.1036 ±0.005
	H	24	[e]		0.15 ±0.05	1.32*** ±1.66		0.10 ±0.01	0.60*** ±0.57	0.0533[f]
	T	56	[e]		1.60 ±1.51	3.73 ±2.48		0.51 ±0.37	1.87* ±1.13	0.0883* ±0.005

[a] C, control; H, hesperidin; T, taxifolin.
[b] Relative germination index.
[c] Fresh weight of five seedlings on 7th day.
[d] *$0.01 < P < 0.08$, ** $0.001 < P < 0.01$, ***$P < 0.0001$.
[e] No germination on 2nd day.
[f] Only five seedlings available.

cies should be significant since this weed is perennial and thus would maintain a sufficient supply of these compounds in addition to other phenolic acids that are also inhibitory to growth and establishment of seedlings.

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