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Full Length Research Paper

Allelopathic effects of *Artemisia princeps* and *Launae sonchoids* on rhizospheric fungi and wheat growth

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The allelopathic effect of *Artemisia princeps* L. *and Launae sonchoids* L. from Taif Governorate, Saudi Arabia was measured in terms of germination rate and radicle length of a bread wheat variety 'Ariana' (*Triticum aestivum* L.). Diluted extracts of shoot systems were applied to seeds of the test plant. The allelopathy of *A. princeps* and *L. sonchoids* varied with the concentration and type of species. The concentrated extracts (3.75 and 5% w/v.) were phytotoxic to germination of wheat. The reduction in the germination percentage reached 15 to 25% and 15 to 30% by treatment with *A. princeps and L. sonchoids* at concentration of 75 and 100% respectively after 8 days of treatment. The aqueous extract of Artemisia at 25% caused an increase in the root and shoot growth of the wheat seedlings in comparison with that of *L. sonchoids* and control. However, the growth of root and shoot gradually decreased with increasing extract concentration (50 to 100%). The biomass production parallel with that of root and shoot lengths were activated at low concentration and inhibited at high ones. The frequency and type of fungal species varies with different plants. The number of fungal population (CFU) on the rhizosphere of *L. sonchoides* was 562 colony/g, while it was 469 colony/g. in the rhizosphere of *A. princeps.* Ten different fungal genera were isolated from the rhizosphere of each of the two plants, the genus Aspergillus was the most frequent followed by Monilia, Rhizoctonia and Rhizopus.

Key words: Allelopathic, *Artemisia princeps*, *Launae sonchoids*, rhizospheric fungi, wheat growth.

INTRODUCTION

Allelopathy is an important mechanism of plant interference mediated by the addition of plant-produced phytotoxins to the plant environment and competitive strategy of plants. Allelopathy involving secondary metabolites is produced by plants, micro-organisms, viruses, and fungi that influences growth and development of biological systems (Oussama, 2003). Allelochemicals are released from plant tissue in a variety of ways including emission of volatile substances from living plant parts, exudation from roots, or leaching from above ground parts by rain, dew, fog, etc. (Ben et al., 2001). Allelopathic effect of *Avena fatua* L., *Cyperus rotundus* L., *Polygonum hydropiper* L., and *Solanum nigrum* L. were examined on seedling growth of certain commonly used varieties of wheat (*Triticum aestivum* L.) in the Tarai region of U.P. state (Chaves and Escudero, 2006).

Many researchers have found that inhibitory substances involved in allelopathy are terpenoids and phenolic substances (Alexa et al., 2004; Chaves and Escudero, 2006; Khanh et al., 2007). A wide array of biologically active constituents are produced by plants in the genus Artemisia (Regina and Belz, 2007). The volatile essential oil of Artemisia species resulted in reduction of seedling survival (Bertholdesson, 2005) . The volatile oil of *Artemisia princeps* and *Launae sonchoids* have been reported to have several biological activities, notably antibacterial, antifungal and anti-oxidative properties (Kil and Yun, 1992). Monoterpene vapours may cause anatomical and physiological changes in plant seedlings and exposure to volatile terpenes can lead to accumulation of lipid globules in the cytoplasm, reduction in organelles including mitochondria and disruption of membranes surrounding mitochondria and nuclei (Monari et al., 2005). The root tip cells subjected to the alkaloids gramine and hordenine caused damages to the cell walls, disorganization of organelles, increase cell vacuoles, and the appearance of lipid and globules, showing food

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reserves (Rasmussen et al., 2004). Large amounts of monoterpene hydrocarbons and/or sesquiterpenes are found to lower the antimicrobial activity of essential oils (Alexa et al., 2004).

Many microorganisms live in soil, but even more (up to 100 times more) live close to the roots of plants (Amal et al., 2003). Information on the rhizospheric fungi of various cultivated and desert plants in different localities in the world have been provided by several investigators (Pandey and Palni, 2007; Pandey et al., 2001; Pinton et al., 2001; Whipps, 2001; Ismail et al., 2003; Keiko, 2005; Sing et al., 2007). Saudian soil is infested with many soil-borne fungi. 25 genera and 68 species, in addition to one variety of each of *Asperigillus chevalieri, Aspergillus flavus* and *Aspergillus nidulans* were isolated from 40 soil samples collected from desert in Saudi Arabia on 5% sodium chloride-Czapek agar (Abdel-Hafez, 1981). *Aspergillus niger, A. flavus, A. nidulans, Phoma glomerata, Phoma humicola, Fusarium oxysporum, Penicillium chrysogenum, Mucor racemosus, Drechslera spicifera* and *Stachybotrys chartarum* were isolated from different dust samples of Taif, Saudi Arabia (Abdel-Hafez and Shoreit, 1985). Altalhi (2004) recorded fifteen fungal species belonging to ten genera from the rhizosphere of some Taif plants.

The present work aimed to determine the allopathic effect of *A. princeps* var. *orientalis* and *L. sonchoids* water extracts on wheat (*T. aestivum* L.) germination and growth and on rhizospheric fungi of the two plants.

MATERIALS AND METHODS

A. princeps var. *orientalis* and *L. sonchoids* were collected from the desert plains at Taif, Saudi Arabia in March 2008. Whole plants were pulled out of the field at the stage of flowering development. The plants were gently washed with distilled water, dried between two paper towels. The plant materials were chopped into 1 cm long pieces and dried at 50°C for 24 h. The components were soaked in distilled water for 24 h at 200 rpm at the rate of 5 g fresh weight per 100 ml distilled water (5% w/v). Each extract was filtered through four layers of cheese cloth, Whatman filter paper under vacuum and stored at 5°C. Dilutions were made of the original extracts as:

C = distilled H_2O
A_1= extract, 1.25% w/v.
A_2= extract, 2.5% w/v.
A_3= extract, 3.7 5% w/v.
A_4= extracts, 5% w/v.

Seeds were surface sterilized with a 5% aqueous solution of sodium hypochlorite for 2 min, rinsed five times with distilled water and dried between two paper towels. Twenty-five seeds were placed in each Petri dish containing agar and placed in the dark at 25°C. After 2, 4, 6, and 8 days, germination was counted and central radicle length were measured and recorded. Seeds were considered germinated when the radicle extended through the seed coat.

Isolation and identification of rhizospheric fungi

Rhizospheric fungi were isolated from soil by dilution plate method (Hanlin and Ulloa, 1979). Several dilutions (0.1, 0.01, 0.001 and 0.0001 ml) were prepared and used for isolating rhizospheric fungi on potato dextrose medium. Fungi were purified and identified on the basis of morphological and physiological characteristics (Alexopouls and Mims, 1979; Burgess et al., 1988; Domsch et al., 1980; Klich and Pitt, 1988; Pitt, 1988). The total fungal counts were determined as colonies forming unite per gram soil (CFU/g).

The experimental design was a complete randomized design with four replicates. All experimental data were subjected to analysis of variance by using SAS (1985) and Fisher's protected least significant difference (LSD) at the 5% level of probability (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

A. princeps and *L. sonchoids* shoot extracts significantly affected germination of wheat seeds (Table 1). Results obtained reavealed that, *A. princeps* and *L. sonchoids* allelopathy takes the form of heterotoxicity, depressive to wheat. The germination percentage of wheat grain decreased by 50 and 35% due to *A. princeps* extract treatment at concentration of A_4 and A_3 respectively, after 2 days. The inhibition decreased to 25 and 15% after 8 days. Treatment with A_1 extract cleared un-significant effect on the germination of bread wheat compared with control in case two type of extracts.

Root and shoot lengths, were measured under both normal and treated conditions (Table 2). The lengths were gradually decreased with the increasing extract concentrations as compared to control. The reduction in the lengths reached 60 to 75% by treatment with Artemisia at concentration of A_3 and A_4, respectively; meanwhile, the reduction reached 65 to 82% by treatment with Launae at concentration of A_3 and A_4, respectively. Responses of wheat to the treatments suggested that, tillage affected solubilization of allelopathic compounds from *A. princeps and L. sonchoids*. Allelopathic compounds were tilled into the soil and solubilized rapidly by soil moisture from rainfall (Roth et al., 2000). The high concentration treatments showed a significant effect on the germination percentage of wheat, as well as root and shoot lengths. The maximum shoot and root lengths as compared to control was estimated at (A_1) 25% treatment.

Water contents and degree of succulence were significantly lower in the A_1 groups as compared with the C group. This decrease persisted up to the eight day, (Table 3) . The treatment with A_2 and A_3 of extracts increased the water contents, as well as degree of succulence of the wheat root and shoot. Meanwhile, in the A_4 extract the water contents increased in the shoot and decreased in the root samples. Priming treatments significantly affected fresh and dry weight of seedlings (Table 4). Fresh weights of seedlings was drastically decreased due to high concentration of *A. princeps and L. sonchoids* extracts, meanwhile low concentration improved fresh and dry weight of seedlings as compared with control. The reduction of fresh and dry weight reached 64% at wheat root seedlings treated with A_4

Table 1. Effects of diluted extracts (%) of *Artemisia princeps and Launae sonchoids* shoots on germination (%) of bread wheat.

Treatments	Time (days)							
	2		4		6		8	
	A. princeps	***L. sonchoids***	***A. princeps***	***L. sonchoids***	***A. princeps***	***L. sonchoids***	***A. princeps***	***L. sonchoids***
Control	60	60	96	96	100	100	100	100
A_1	53	51	89	84	93	91	100	98
A_2	44	40	78	75	87	84	95	92
A_3	40	36	72	68	82	79	86	83
A_4	25	21	44	41	58	55	75	71
LSD (5%)	1.68	1.60	0.91	0.9 5	0.75	0.70	0.75	0.64

Means within a column followed by significantly different according to Fisher's protected least significant difference (LSD) (P<0.05). A_1= extract, 1.25% w/v.; A_2= extract, 2.5% w/v.; A_3 = extract, 3.7 5% w/v.A_4 = extracts, 5% w/v.

Table 2. Effects of diluted extracts (%) of *Artemisia princeps and Launae sonchoids* shoots on shoots, root and shoot lengths (cm) of wheat at day 8.

Treatments	Root		Shoot	
	A. princeps	***L. sonchoids***	***A. princeps***	***L. sonchoids***
Control	5.2	5.2	14.3	14.3
A_1	4.9	4.2	15.2	14.2
A_2	3.7	3.2	12.7	11.7
A_3	2.8	2.1	11.2	10.3
A_4	1.9	1.6	10.1	9.6
LSD (5%)	0.56	0.43	1.2	1.1

Means within a column followed by significantly different according to Fisher's protected least significant difference (LSD) (P<0.05). A_1 = extract, 1.25% w/v.; A_2 = extract, 2.5% w/v.; A_3 = extract, 3.7 5% w/v. A_4 = extracts, 5% w/v.

Artemisia extract meanwhile, the reduction reached 69% at wheat root seedlings treated with A_4 Launae extract. The allelopathic compounds released from *A. princeps and L. sonchoids* were introduced into the soil and were presumably degraded over time.

Although, emergence was delayed by tilled sorghum in many cases, the marked ability of wheat to compensate for differences in seedling development probably also contributed to the absence of any effect on grain yield (Paulsen, 1987). Solubilization and leaching of allelopathic compounds from Artemisia depended on precipitation, as was the case with Artemisia incorporated into soil (Purvis, 1990). The amount, intensity and duration of precipitation and the air temperature probably influenced the leaching pattern of allelopathic compounds, just as they affected loss of other soluble constituents from senescing tissue (Noodén, 1980). The delayed effect of Artemisia also suggested that allelopathic compounds degraded slowly, if at all, in non-incorporated compared with their rapid degradation in soil (Guenzi et al., 1967). Some differences in responses of the wheat cultivars to allelopathy were evident during different growth stages, but none of the cultivars was resistant at both the seedling and grain filling stages. If they were available, cultivars that resist allelopathy during the latter stage might be combined with

Table 3. Water contents (W.C) and degree of succulence (D.Su.) of root and shoot systems of wheat under the effect of diluted extracts (%) of *Artemisia princeps and Launae sonchoids* shoots after 8 days.

Treatments	Shoot				Root			
	W.C %		D. Su.		W.C %		D. Su.	
	A. pri.	*L. son.*	*A. pri.*	*L. son.*	*A. pri.*	*L. son.*	*A. pri.*	*L. son.*
Control	66.03	66.03	2.94	2.94	70.93	70.93	3.44	3.44
A_1	55.17	58.14	2.23	2.63	70.83	73.89	3.43	3.52
A_2	75.00	77.32	4.00	4.20	85.90	88.95	3.72	3.83
A_3	69.70	72.41	3.30	3.80	88.89	90.69	3.74	3.90
A_4	63.64	66.64	2.75	2.95	88.24	91.44	3.09	3.98
LSD (5%)	1.32	1.82	0.08	0.10	2.67	2.97	0.07	0.13

Means within a column is significantly different according to Fisher's protected least significant difference (LSD) (P<0.05). A_1= extract, 1.25% w/v.; A_2= extract, 2.5% w/v.; A_3= extract, 3.7 5% w/v.A_4= extracts, 5% w/v.

Table 4. Fresh and dry weight of root and shoot systems of wheat under the effect of diluted extracts (%) of *Artemisia princeps and Launae sonchoids* shoots after 8 days.

Treatments	Shoot (g/plant)				Root (g/plant)			
	Fresh weight		Dry weight		Fresh weight		Dry weight	
	A. pri.	*L. son.*	*A. pri.*	*L. son.*	*A. pri.*	*L. son.*	*A. pri.*	*L. son.*
Control	0.053	0.053	0.018	0.018	0.086	0.086	0.025	0.025
A_1	0.058	0.046	0.026	0.023	0.096	0.076	0.028	0.024
A_2	0.044	0.039	0.011	0.010	0.078	0.066	0.021	0.019
A_3	0.033	0.031	0.01	0.009	0.063	0.060	0.017	0.016
A_4	0.022	0.019	0.008	0.008	0.034	0.029	0.011	0.010
LSD (5%)	0.015	0.015	0.004	0.004	0.08	0.06	0.002	0.002

Means within a column is significantly different according to Fisher's protected least significant difference (LSD) (P<0.05). A_1= extract, 1.25% w/v.; A_2= extract, 2.5% w/v.; A_{3b} = extract, 3.7 5% w/v. A_4 = extracts, 5% w/v.

suitable tillage of Artemisia residue for highest yields of wheat.

Our results suggest that the effect of Artemisia residue on the following wheat crop depends in large part on the degree of decomposition of the stover before the wheat is planted. Although, it was not investigated here, chopping the Artemisia stover, finely also might accelerate decomposition. The beneficial effects of adequate soil moisture (Purvis, 1990) also suggest that irrigation of dry soil could promote decomposition and lessen allelopathy from Artemisia on wheat. The merits of practices that reduce allelopathy from Artemisia must be weighed against the soil-conserving benefits of not tilling the stover. Tillage should only be considered when the soil is not subject to erosion from water and wind. Artemisia residue on highly erodible soil should not be tilled.

We found that the number of fungal population (CFU) differ with different plant, and the number of fungal population on the rhizosphere of *L. sonchoides* was 562 colony/g, while the number of fungal population on the rhizosphere of *A. princeps* was 469 colony/g. Our results agreed with El-abyad et al*.* (1982). They concluded that the microbial counts in the rhizosphere of the desert halophytic plants were considerably stimulated and the degree of such stimulation varies according to the species and prevailing environmental conditions. Also, our results are in accordance with that of Altalhi (2004). He reported that the highest number of fungal colonies per gram of Taif soil rhizosphere was recorded on *Artemisia judaica* (320.4 colony/g), and the lowest number on *Desmostachya bipinnata* (1.6 colony/g). Also, our results agreed with that of Abou-Zeid and Abd El-Fattah (2007). They concluded that, the highest number of fungal populations of some Taif plants were found in the rhizosphere of *Asphodelus aestivus* (131 colony /g), followed by *Pulicaria crispa* (83 colony/g) and the lowest number was reported on the rhizosphere of *Argenone ochroleuce* (74 colony/g). This difference in total fungal counts (CFU) may be due to the difference in rhizodeposition, the place and type of plant soil (Evenari et al., 1971; Mahmoud and El-Tom, 1985; Abou-Zeid and Abd El-Fattah, 2007; Abou- Zeid and Altalhi, 2009).

From Table 5, we concluded that the most frequent fungal genera on the rhizosphere of Artemisia princeps

Table 5. Fungal rhizospheres of *Artemisia princeps* and *Launae sonchoids* plants.

Fungal rhizosphere of *Artemisia princeps*	Frequency	Fungal rhizosphere of *Launae sonchoids*	Frequency
Monilia acremonium	+++	*Pythium intermedium*	++
Rhizopus oryzae	+++	*Monacrosporium bembicodes*	++
Mucor microsporus	+++	*Cladosporium herbarum*	+
Trichoderma viride	++	*Trichoderma viride*	+
Aspergillus candidus	+	*Monilia acremonium*	+++
Aspergillus sydawii	+	*Fusarium solan*i	+
Aspergillus niger	+++	*Rhizoctonia solani*	+++
Aspergillus sulphureus	+++	*Alternaria alternata*	++
Mortierella isabellina	+	*Aspergillus niger*	+
Mycelia sterilia	++	*Rhizopus oryzae*	+++

plant were Aspergillus (4 species), while the other genera (Monilia, Rhizopus, Mucor, Trichoderma, Mortierella and *Mycelia sterilia*) were reported only by one species. While *Pythium intermedium*, *Monacrosporium bembicodes*, *Cladosporium herbarum*, *Fusarium solani* and *Alternaria alternata* were found only on the rhizosphere of *L. sonchoides* . *Mucor microsporus*, *Aspergillus candidus*, *Aspergillus sydawii*, *Aspergillus sulphureus*, *Mortierella isabellina* and *M. sterilia* were reported only in the rhizosphere of *A. princeps*. These results agreed with Abdel-Hafez and Shoreit (1985), they isolated 70 species and 31 genera from 20 dust samples of Taif, Saudi Arabia and the most common genera were Aspergillus, Fusarium, Penicillium and Mucor. Abou-Zeid and Abd El-Fattah (2007) studied the rhizosphere of four dominant plants in Taif governorate namely *A. ochroleuce*, *A. aestivus*, *Peganum harmala* and *P. crispa* and found that the most frequent genera were Aspergillus (7 species) followed by Fusarium (4 species), Alternaria and Rhizopus (1 species). From these genera the most abundant fungal species in the plant rhizospheres were *A. niger* (P= 83.3%), Fusarium sp1 (P= 75%), *Aspergillus ochraceus* (P= 66.7) and *Alternaria alternata* (P=58.3%) and the lowest abundant fungal species were *Fusarium acuminatum*, *Fusarium lateritium*, *Fusarium* sp2, *Fusarium poae* and *Phoma pomorum* (P= 8.3). Maghazy et al. (2008) isolated *Pythium spinosum* and controlled it by *Aspergillus carneus, Aspergillus cervinus, A. sulphureus, Penicillium funiculosum, Penicillium Islandicun, Penicillium nigricans, Chaetomium globosum, Paecilomyces lilacinus* and *P. pomorum* from the rhizosphere soil of Egyptian clover, which support our results. Our results in Table (5) concluded that, the frequency and type of fungal species varies with different plants. These results agreed with Shindia and Abdel-Fattah (1994); Pandey et al. (2001); Jain and Gupta (2002); Abou-Zeid et al. (2008).

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