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Full Length Article

Allelopathic Interaction of Wheat (*Triticum aestivum*) and Littleseed Canarygrass (*Phalaris minor*)

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Abstract

Allelopathy is a plant-plant or plant-microbe interaction in which one plant produces secondary metabolites i.e., allelochemicals, which may influence the chemistry of its rhizosphere and affect the growth of neighbouring plants. Management of weeds using synthetic chemicals is environmentally hazardous. Allelopathy is cost effective and eco-friendly natural phenomenon that can be manipulated for weed management. An experiment was conducted to study the allelopathic interaction of wheat and littleseed canarygrass using the equal-compartment-agar method. Wheat cultivars and promising lines (Faisalabad-08, Lasani-08, Shafaq-06, Sehar-06, Miraj-08, Farid-06, Chakwal-50, V-04178, V-05066 and V-05082) were used to study their allelopathic interaction with littleseed canarygrass. Each wheat cultivar was grown alone and in association with littleseed canarygrass in glass beakers containing water agar solution, kept in a growth cabinet maintaining 25/18ºC day and night temperature, respectively; 13/11 h light/dark period. Littleseed canarygrass was also grown alone as control for comparisons. Results revealed that wheat cultivars had differential allelopathic inhibition activity against littleseed canarygrass through the production of phenolic compounds. Maximum inhibition in root length (54%), shoot length (59%), root dry weight (60%) and shoot dry weight (55%) of littleseed canarygrass was recorded when grown in association with wheat cv. Shafaq-06, while all these growth parameters were less in association with cv. Sehar-06. Significant increase in production of total soluble phenolics was also observed in root and shoot of all wheat cultivars when grown in association with littleseed canarygrass as compared to when grown alone. In conclusion, cv. Shafaq-06 was found strongly allelopathic against littleseed canarygrass. © 2015 Friends Science Publishers

Keywords: Allelopathy; Total soluble phenolics; Littleseed canarygrass; Wheat

Introduction

Wheat (*Triticum aestivum* L.) is the major cereal and staple food of Pakistan. The reduction in yield of wheat due to weed infestation has been estimated up to 30% (Ahmad *et al.,* 2005). Among many weeds, littleseed canarygrass (*Phalaris minor* Retz.) is widespread in rice-wheat cropping system of Pakistan. Due to its strong competitive ability with wheat, it can cause 10-50% yield losses (Ranjit *et al.,* 2006).

Allelopathy refers to the beneficial or harmful effects of one plant on another by the production of certain chemicals from various plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and manmade systems (Gibson and Liebman, 2003). Allelochemicals appear to alter a variety of physiological processes like cell division, cell differentiation, ion and water uptake, water status, phytohormone metabolism, respiration, photosynthesis and enzymatic functions in plants (Singh *et al.,* 2003; Belz and Hurle, 2004). Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids etc. (Weston and Duke, 2003). It can be utilized efficiently for weed control with the aim of environmental safety as an alternative of chemical herbicides (Tabaglio *et al.,* 2008; Farooq *et al*., 2011, 2013). If a weed species is suppressed by crop plants during its seedling establishment period by the production of certain allelochemicals, crop plants will gain an advantage over weed subsequently leading to a better approach towards effective weed management.

Various crops possess allelopathic potential, including wheat (Lam *et al*., 2012), oat (De Bertoldi *et al.,* 2009), and rice (Dilday *et al.,* 2001). Wheat has been examined extensively for its differential allelopathic potential among accessions. It contains allelochemicals like phenolic compounds that can inhibit weed growth under field conditions (Liebl and Worsham, 1983; Wu *et al.,* 1999, 2000). Durum wheat (*Triticum durum* L.) also showed inhibitory effects when its leaf, stem and root water extracts were applied on barley and bread wheat (Oueslati, 2003; Krogh *et al.,* 2006).

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In spite of extensive research on wheat allelopathy across the globe, no significant work has been done in Pakistan regarding this aspect of allelopathy, so this experiment evaluated the allelopathic potential of indigenous wheat cultivars and promising lines against littleseed canarygrass and also the allelopathic effects of littleseed canarygrass on all these wheat cultivars and promising lines.

Materials and Methods

Chemicals

All reagents used were at least analytical reagent (AR) grade including ethanol (Merck Ltd.), acetone (BDH Chemicals, UK), sodium hypochlorite (Fluka, USA), Folin-ciocaltue reagent (Unichem, USA), gallic acid (Fluka, USA), agar lab (Lab M. Limited, UK) and sodium carbonate (MP Biomedicals LLC, France).

Seed Collection

Seeds of wheat cultivars and promising lines (Faisalabad-08, Lasani-08, Shafaq-06, Sehar-06, Miraj-08, Farid-06, Chakwal-50, V-04178, V-05066 and V-05082) were obtained from Wheat Research Institute, AARI, Faisalabad-Pakistan and of littleseed canarygrass from Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan during 2010. Wheat and littleseed canarygrass seeds were surface-sterilized by soaking in 70% ethanol for 2.5 min, followed by rinsing 4 times in sterilized distilled water, and then soaked in sodium hypochlorite (2.5%) solution for 15 min, which was followed by 5 time rinsing in sterilized distilled water.

Germination Bioassay

Surface-sterilized seeds of each wheat cultivar and of littleseed canarygrass were soaked in sterilized water in light at 25°C for 24 h and then rinsed with sterilized water. Then, 600 seeds of littleseed canarygrass and 200 of each wheat cultivar were incubated in light at 25°C and 22°C, respectively for 48 h in glass house

General Description of Equal-compartment-agar Method (ECAM)

A glass beaker (1000 mL) containing 70 mL of 0.3% water agar (no nutrients) was autoclaved. Germinated seeds of each wheat cultivar (16), selected uniformly in size were aseptically sown on one-half of the agar surface with the embryo up and on the other half 16 seeds of littleseed canarygrass were sown. The beakers were covered with aluminium foil (from the top) and wrapped with para-film, placed in a controlled environment growth cabinet with 25/18ºC day and night temperature, respectively; 13/11 h light/dark period. Seedlings were removed from agar media and 10 seedlings of each wheat cultivar and of littleseed canarygrass were taken for measurement of root length, shoot length, root and shoot dry weight and total soluble phenolics after 10 days of growth (Wu *et al.,* 2000). The growth of wheat and littleseed canarygrass grown alone served as the control.

Determination of Total Soluble Phenolics

Plant fresh tissue (0.5 g) of both roots and shoots of wheat and littleseed canarygrass was collected and extracted with pestle and mortar using 80% acetone and 10 mL final volume was made. Standards of 50, 100, 200 and 500 µL were prepared from 1000 µL stock solution of gallic acid and by running on spectrophotometer at 760 nm standard curve was drawn. A 20 µL of extracted solution was placed in a 5 mL test tube with 1580 µL of water and 100 µL of Folllen-Ciocaltue reagent were added. After 30 sec, 300 µL of sodium carbonate (20%) was added to the sample mixture. The samples after treating with all these reagents were kept at room temperature for 2 h, then were run on spectrophotometer against pure water sample with no extract at 760 nm and absorbance was recorded. The concentration of total soluble phenolics was calculated using the standard curve.

Statistical Analysis

The experiment was established in a completely randomized design with three replications. The data were subjected to analysis of variance using MSTAT-C. The mean differences were separated using LSD test at 0.05 probability level. Percentage inhibition of both littleseed canarygrass and wheat was calculated as [(Control –treatment)/Control]× 100 (Wu *et al.,* 2000).

Results

Allelopathic Effect of Different Wheat Cultivars on Littleseed Canarygrass

Results indicated that all wheat cultivars and promising lines suppressed the growth of littleseed canarygrass. When littleseed canarygrass was grown alone, highest root length (6.20 cm) was recorded, than with littleseed canarygrass when grown in association with wheat, suppression in root length was observed as compared to control *i.e.,* littleseed canarygrass alone (Table 1). Maximum suppression in root length of littleseed canarygrass was recorded when grown with Shafaq-06 (54%) followed by Faisalabad-08 (50%), Chakwal-50 (43%), Lasani-08 (40%), Miraj-08 (33%), Farid-06 (29%), V-04178 (24%), V-05066 (18%), V-05082 (16%) while, Sehar-06 showed the least inhibitory effect on the root length of littleseed canarygrass (7%).

Wheat cultivars also inhibited the shoot length of littleseed canarygrass. Maximum reduction in shoot length was observed when littleseed canarygrass was grown in association with Shafaq-2006 (59%) followed by Faisalabad-2008 (56%), Chakwal-50 (49%), Lasani-2008

(45%) and Miraj-2008 (41%) (Table 1). Similarly maximum root dry weight of littleseed canarygrass was recorded when grown with Shafaq-2006 i.e., 1.67 mg, (60%) followed by Faisalabad-08, 1.80 mg (56%), Chakwal-50 (50%), Miraj-08 (47%), Lasani-08 (46%), Farid-08 (40%), V-05066 (35%), V-05082 (32%), V-04178 (31%) and there was the minimum reduction in root dry weight of littleseed canarygrass when grown in association with Sehar-06 (21%) (Table 1). Shoot dry weight of littleseed canarygrass was observed 5.37 mg when it was grown alone and Shafaq-06 reduced it to 2.40 mg (55%), followed by, Faisalabad-08 (50%), Chakwal-50 and V-04178 (45%), Miraj-08 (42%), Lasani-08 (41%), V-05066 (39%), Farid-06 (38%), V-05082 (32%) and Sehar-06 (23%). All wheat genotypes reduced the total soluble phenolic contents in littleseed canarygrass root and shoot with a range of 5-53% and 8-45% for root and shoot respectively. Wheat cultivar Sahfaq-06 (53%) was followed by Faisalabad-08 (46%), Chakwal-50 (39%), Lasani-08 (36%), Miraj-08 and Farid-06 (30%), V-04178 (17%), V-05066 (12%), V-05082 (10%), suppressed root phenolics but Sehar-06 again showed poor ability to suppress total soluble phenolics in roots of littleseed canarygrass *i.e.* 5%. The suppression in root phenolics by other cultivars was less than Shafaq-06 and Faisalabad-08, but more than Sehar-06 (Table 2).

Similarly, shoot phenolics of littleseed canarygrass was also suppressed maximally by Shafaq-06 (45%) followed by Faisalabad-08 (42%), Chakwal-50 (38%), Lasani-08 (36%), Miraj-08 (29%), Farid-06 and (24%), V-04178 (21%), V-05066 (17%), V-05082 (13%) and Sehar-06 (8%) (Table 2).

Allelopathic Effect of Littleseed Canarygrass on Different Wheat Cultivars

Littleseed canarygrass suppressed all wheat cultivars. However, wheat genotypes showed differential behaviour against littleseed canarygrass. Sehar-06 had the maximum inhibition in root length (19%) and shoot length (17%). However Shafaq-06 had 7% and 15% inhibition in root and shoot length respectively whereas Faislabad-08 recorded 9% root inhibition and 14% shoot inhibition when grown in association with littleseed canarygrass. There was another interesting observation i.e., 3% shoot inhibition in V-05066 than can be inferred as ability of V-04178 to tolerate littleseed canarygrass up to some extent (Table 3).

There was maximum effect of littleseed canarygrass on Sehar-06 that showed 27% reduction in root dry weight in interaction as compared to grown alone and was followed by Farid-06 and V-05082 with 18% suppression,

Table 1: Allelopathic effect of different wheat genotypes on the growth of littleseed canarygrass

Varieties/ genotypes	Root length (cm)	Shoot length (cm)	Root dry weight (mg)	Shoot dry weight (mg)
Canarygrass alone	6.20 a	10.27 a*	4.13 a	5.37 a
Shafaq-06 + canarygrass	2.87 g(54)*	4.20 d(59)	1.67 f(60)	2.40 g(55)
Miraj-08+ canarygrass	4.13 e(33)	6.03 bcd(41)	2.20 e(47)	3.13 de(42)
Sehar-06+ canarygrass	5.73 b(07)	7.33 b(29)	3.27 b(21)	4.13 b(23)
Farid-06+ canarygrass	4.40 e(29)	6.46 bcd(37)	2.47 d(40)	3.33 d(38)
V-04178+ canarygrass	4.73 d(24)	6.37 bcd(38)	2.87 c(31)	2.93 e(45)
Faisalabad-08+ canarygrass	3.13 g(50)	4.57 cd(56)	1.80 f(56)	2.67 f(50)
V-05082+ canarygrass	5.23 c(16)	7.00 bc(32)	2.80 c(32)	3.67 c(32)
V-05066+ canarygrass	5.10 c(18)	6.53 bcd(36)	2.67 cd(35)	3.27 d(39)
Chakwal-50+ canarygrass	3.53 f(43)	5.20 bcd(49)	2.07 e(50)	2.93 e(45)
Lasani-08+ canarygrass	3.70 f(40)	5.63 bcd(45)	2.20 e(47)	3.13 de(42)
LSD (0.05)	0.266	2.549	0.262	0.248

* Values in parenthesis indicate percent inhibition over control (canarygrass alone); Means with the same letter within a column do not differ at p 0.05

Table 2: Total soluble phenolics in littleseed canarygrass when grown alone and in association with different wheat genotypes

Varieties/ genotypes	Root (mg kg^{-1})	Shoot (mg kg^{-1})
Canary grass alone	3.00a	2.28 a
Shafaq-06 + canarygrass	1.44 k(53)*	1.27 k(45)
Miraj-08+ canarygrass	2.09 g(30)	1.61 g(29)
Sehar-06+ canarygrass	2.84 b(05)	2.10 b(08)
Farid-06+ canarygrass	2.30 f(23)	1.73 f(24)
V-04178+ canarygrass	2.50 e(17)	1.80 e(21)
Faisalabad-08+ canarygrass	1.63 j(46)	1.33 j(42)
V-05082+ canarygrass	2.71c(10)	1.98 c(13)
V-05066+ canarygrass	2.63 d(12)	1.89 d(17)
Chakwal-50+ canarygrass	1.82 i(39)	1.41 i(38)
Lasani-08+ canarygrass	1.93 h(36)	1.46 h(36)
LSD (0.05)	0.068	0.023

* Values in parenthesis indicate percent decrease over control (canarygrass alone); Means with the same letter within a column do not differ at p 0.05

Table 3: Allelopathic effect of littleseed canarygrass on root and shoot length of different wheat cultivars/ genotypes

Varieties	Root length (cm)			Shoot length (cm)		
	Wheat alone	Wheat+littleseed canarygrass	% decrease	Wheat alone	Wheat+littleseed canarygrass	% decrease
Shafaq-06	12.12 a	11.20 a	07	20.67 a	17.57 a	15
Miraj-08	8.50 e	7.40 e	13	16.33 d	15.20 c	07
Sehar-06	6.02 h	4.87 h	19	12.30 h	10.17 f	17
Farid-06	7.70 f	6.60 f	14	15.68 de	14.67 c	06
V-04178	7.38 fg	6.40 fg	13	14.77 ef	13.17 d	11
Faisalabad-08	11.12 b	10.10 b	09	19.00 b	16.43 b	14
V-05082	7.17 g	6.20 g	14	13.00 gh	11.50 e	12
V-05066	7.17 g	6.20 g	14	13.77 fg	13.33 d	03
Chakwal-50	10.67 c	9.40 c	12	17.83 c	15.50 c	13
Lasani-08	9.57 d	8.47 d	11	17.37 c	15.33 c	12
LSD (0.05)	0.349	0.303	-----	1.011	0.837	-----

Means with the same letter within a column do not differ at p 0.05

Table 4: Effect of littleseed canarygrass on root and shoot dry weight of different wheat cultivars/genotypes

Varieties	Root dry weight (mg)			Shoot dry weight (mg)		
	Wheat alone	Wheat + littleseed canarygrass	% decrease	Wheat alone	Wheat + little seed canary grass	% decrease
Shafaq-06	6.20 a	5.73 a	08	12.10 a	10.53 a	13
Miraj-08	4.17 e	3.67 e	12	10.40 e	8.60 e	17
Sehar-06	2.90 i	2.13 h	27	8.17 j	6.57 g	20
Farid-06	3.80 f	3.13 f	18	10.03 f	8.17 d	19
V-04178	3.40 g	3.07 f	10	9.63 g	7.83 de	19
Faisalabad-08	5.90 b	5.00 b	15	11.87 b	10.23 a	14
V-05082	3.03 hi	2.47 g	18	8.77 i	7.03 f	20
V-05066	3.23 gh	3.13 f	03	9.10 h	7.63 e	16
Chakwal-50	5.47 c	4.73 c	14	10.97 c	9.13 b	17
Lasani-08	5.00 d	4.20 d	16	10.57 d	8.80 bc	17
LSD (0.05)	0.248	0.232		0.164	0.347	

Means with the same letter within a column do not differ at p 0.05

while the minimum effect of littleseed canarygrass was observed on V-04178, which showed only 3% reduction in root dry weight when grown in association with littleseed canarygrass as compared to grown alone (Table 4).

In case of shoot dry weight of different wheat cultivars, Sehar-06 and V-05082 were affected maximum by littleseed canarygrass with 20% reduction in shoot dry weight. Next to it were Farid-06 and V-04178 with 19% reduction in shoot dry weight and minimum reduction (13%) was recorded in case of Shafaq-06 when grown with littleseed canarygrass. However, there was more reduction in root dry weight as compared to shoot dry weight in all wheat cultivars when grown in association with littleseed canarygrass (Table 4).

Total soluble phenolics were determined in both wheat roots and shoots for all the treatments. There was gradual increase in total soluble phenolics in root as well as shoot when grown with littleseed canarygrass. Stress conditions created by littleseed canarygrass could have caused this increase.

There was 131% increase in root phenolics of Shafaq-06, when grown in association with littleseed canarygrass than when grown alone and Faisalabad-08 had an increase of 120%. All other genotypes showed considerable increase (76-117%) except Sehar-06 that showed only 36% increase when grown in association with littleseed canarygrass. Shafaq-06 also showed the maximum increase in its shoot phenolics (42%) followed by Faisalabad-08 (31%). Sehar-06 had also exhibited considerable increase of 20%. However, V-04178 and V-05066 only had an increase of 7%. All other genotypes had an increase of 12-26% (Table 5).

Correlation of Total Plant Phenolics of Wheat Cultivars with Littleseed Canarygrass

Correlation analysis revealed a strong negative relationship between growth indices of littleseed canarygrass and total soluble phenolics in wheat root and shoot (Table 6).

Discussion

Wheat cultivars showed differential allelopathic activity against littleseed canarygrass. Inhibition of the weed through wheat allelopathy is cultivar dependant. Wheat cultivars showed strong variation in their inhibiting ability against annual ryegrass (Wu *et al.*, 1999). Allelopathic inhibition is due to the presence of allelochemicals and major group of allelochemicals in wheat is phenolics (Jensen *et al.*, 2001). Significant variations were observed in wheat cultivars used in present study for their ability to produce phenolic compounds (Table 5). The cultivars having higher total soluble phenolic contents (Shafaq-06 and Faisalabad-08) did more growth inhibition of littleseed canarygrass than the cultivars having lesser amount of total soluble phenolics (Table 3 and 4).

Table 5: Total soluble phenolics in wheat shoot and root when grown alone or in association with littleseed canarygrass

Varieties	Root phenolics (mg kg^{-1})			Shoot phenolics (mg kg^{-1})		
	Wheat alone	Wheat + littleseed canarygrass	% increase	Wheat alone	Wheat + littleseed canarygrass	% increase
Shafaq-06	4.62 a	10.65 a	131	2.78 a	3.95 a	42
Miraj-08	2.83 e	6.13 e	117	2.16 e	2.59 e	20
Sehar-06	1.93 i	2.62 j	36	1.35 j	1.70 j	26
Farid-06	2.50 f	5.39 f	116	2.12 f	2.37 f	12
V-04178	2.37 fg	4.79 g	102	1.94 g	2.08 g	07
Faisalabad-08	4.30 b	9.47 b	120	2.62 b	3.45 b	31
V-05082	2.10 h	3.70 i	76	1.64 i	1.81 i	10
V-05066	2.27 g	4.04 h	78	1.84 h	1.97 h	07
Chakwal-50	3.70 c	7.69 c	108	2.53 c	3.08 c	22
Lasani-08	3.29 d	7.13 d	117	2.33 d	2.78 d	19
LSD (0.05)	0.133	0.135		0.094	0.0933	

Means with the same letter within a column do not differ at p 0.05

Table 6: Correlation for total soluble phenolics and growth indices of littleseed canarygrass and wheat against each other (n = 10)

Phenolics	Littleseed canarygrass			
	Root length	Root dry weight	Shoot length	Shoot dry weight
Total soluble phenolics in wheat	-0.98**	-0.95**	-0.97**	-0.77^{NS}
	Wheat			
Total soluble phenolics in littleseed canarygrass	-0.97**	-0.97**	-0.94**	-0.97**

Moreover, correlation analysis also showed a strong relationship of phenolic compounds and growth indices. Phenolic compounds in each *i.e.* wheat and littleseed canarygrass showed a negative relationship with the growth indices of other (Table 6). It is reported that phenolic compounds as allelochemicals interact with plant metabolic system in various ways that retard the growth of neighbouring species. They increase the cell membrane permeability, which enhances per-oxidation of lipids that result in slowing down the growth or even sometimes death of the plant (Cruz *et al.*, 1998). Phenolics also modify cell ultra-structure, inhibit cell division and cell elongation (Li *et al.*, 1993) and interfere with photosynthesis and respiration by weakening O_2 absorption capacity of plants and destroying chlorophyll contents. It is also believed that they affect stomatal conductance and leaf transpiration in plants (Yu *et al.*, 2003). The growth inhibition of each other might be the result of all or some of these phenomenons that could have happened due to the production of phenolic compounds. When two different species are grown together, they compete with each other for nutrients, light, water and space (Ahmad and Ghafoor, 2004) that can cause stress conditions for either or both the species. Generally, under stress conditions there is more production of allelochemicals in plants like under water stress conditions; there was more production of chlorogenic acid by some plants than normally growing plants (Li *et al.*, 2001). In this study, littleseed canarygrass might have imposed some abiotic stress on wheat plants that caused more production of phenolic compounds by wheat cultivars and these phenolics might have suppressed littleseed canarygrass.

Conclusion

Wheat genotypes Shafaq-06 and Faisalabad-08 are allelopathic against littleseed canarygrass. These genotypes showed good ability to suppress littleseed canarygrass and produced relatively higher amount of total soluble phenolics as compared to other genotypes under laboratory conditions. This is preliminary study, further studies may be planned to characterize the allelochemicals involved.

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