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Wheat Residue Incorporation Modulate Emergence and Seedling Growth of Canary Grass by Affecting Biochemical Attributes and Soil Properties

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Abstract

Residue incorporation greatly modifies the soil chemical properties, and regulates seed germination and subsequent growth of neighboring species by inducing metabolic changes. Such growth regulatory effects often vary among cultivars and the stage of growth of donor plants. Little information is available on the induced biochemical changes in receiver plants and the soil chemical properties when wheat residue collected at different growth stages is incorporated into the soil. Bioassays were conducted to appraise the allelopathic potential of residue (8 g kg^{-1} soil) of hexaploid wheat (*Triticum aestivum* L.) cultivars (Millat-2011, AARI-2011, Lasani-2008 and Faisalabad-2008) collected at tillering (Z-30), anthesis (Z-60) and maturity (Z-90)] against canary grass (*Phalaris minor* Retz.). Mean emergence time of canary grass was prolonged over control by soil incorporation of residue at anthesis and maturity stages of cultivars AARI-2011 and Lasani-2008. Final emergence percentage declined by 13–31% for residue collected at different growth stages. Maximum suppression in shoot (33–51% and 28–53%) and root (34–52% and 28–54%) length and seedling dry biomass (66–88% and 58–86%) of canary grass over control was also recorded under aforementioned treatment combinations. Total chlorophyll contents in canary grass declined in response to soil incorporation of residue at anthesis and maturity stages of all wheat cultivars but an increase was recorded for residue incorporated at tillering stage. Phenolic contents in residue and residue-amended soil increased with advancement in stage of wheat growth. Biochemical bases of phytotoxicity and changes in activities of enzymatic antioxidants in canary grass seedling are discussed. Such information suggests the growth regulatory potential of residue of specific wheat cultivars against weeds of economic significance in wheat based cropping systems and scope for increasing nutrient status of soils.

Keywords: Biochemical attributes; Crop residue; Emergence dynamics, Growth regulation; Soil incorporation

Introduction

Herbicides are commonly used to combat weed menace in wheat for improved quality and quantity of final produce. However, continuous herbicide use has resulted in evolution of resistant weed biotypes and environment pollution (Khaliq *et al*., 2011a). Weed herbicides resistance has emerged as greatest ecological challenge to agriculture (Baucom, 2009) and more than 346 herbicide resistant weed biotypes are reported (Heap, 2010). In wake of sustainable agriculture and negative implications of extensive use of synthetic herbicides, there is need switch from conventional weed management to environmentally friendly approaches. Increasing interest in ecological based weed management has revealed the significance of allelopathy as a tool for sustainable weed management in agro-ecosystems (Albuquerque *et al*., 2011). It is environmentally safe to conserve the available resources and have potential to mitigate the problems raised by synthetic chemicals (Duke *et al*., 2001). Allelopathic effects may be stimulatory or inhibitory depending upon the species involved and threshold concentrations of bioactive allelochemicals. Allelopathic interactions can be used to achieve selective weed control in field crops (Weston and Duke, 2003). In agriculture, the suppressive allelopathic effects can be exploited for sustainable pest and weed control (Khanh *et al*., 2005). It is novel approach offering multiple solutions to overcome problems decreasing food availability under rising global population (Hussain *et al*., 2014).

Wheat is known to possess allelopathic potential against weeds and crops (Khaliq *et al*., 2011b, 2012). Some wheat accessions significantly inhibited the growth of several weeds (up to 75%) comparable with hand weeding (Rizvi *et al*., 2004). Allelopathic effects are species-specific

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and concentration dependent (Wu *et al*., 2000; Belz and Hurle, 2004), and modified owing to their movement, persistence and degradation/transformation in the soil (Inderjit, 2001). Crop allelopathy also varies in response to plant age and different development stages manifested varying levels of different allelochemicals and their relative concentrations (An *et al*., 2003; Iannucci *et al*., 2012). At vegetative stage, allelopathy can suppress weeds through the exudation of allelochemicals into the growth medium by limiting growth and minimizing application of synthetic chemicals. Concentrations of allelochemicals increases in plant's root and shoot at lateral growth stages and affect the emergence and seedling growth of weeds via leaching and residue decomposition (Wu *et al*., 2000).

Canary grass (*Phalaris minor* Retz.) is highly competitive grassy weed of wheat and can reduce grain yield of wheat by 28–34% (Hussain *et al*., 2014). Although native to Mediterranean region, it has now spread to 60 countries worldwide (Singh *et al*., 1999). Rice straw and stubble extract at 10% concentration inhibited the emergence and seedling growth of canary grass (Tamak *et al*., 1994). Adding further, Om *et al.* (2002) reported 42 and 15% inhibition in plant population of canary grass, owing to sunflower (*Helianthus annuus* L.) and dhaincha (*Sesbania aculeate* (Willd.) Pers.) allelochemicals when used as green manure under field conditions. Nevertheless, information regarding the growth regulatory activity of wheat cultivars against canary grass is limited, and the influence of wheat residue collected at different growth stages on canary grass has rarely been investigated. The identification of wheat cultivars with strong allelopathic potential can contribute directly to weed suppression by inclusion into crop rotation, or these can be used in breeding program as selection criterion making future genotypes more competitive against weeds. The pattern of decomposition, and hence, release of allelochemicals with subsequent effects on the germination, growth and chemical attributes of accompanying weeds must be understood for strengthening eco-physiological phenomenon of allelopathic interactions. A number of high yielding wheat cultivars with contrasting morpho-physiological attributes have been released to abridge yield gap in Pakistan. Genotypic variation for allelopathic response of such varieties is yet to be established. Little is known about the induced biochemical changes in canary grass in response to negative effects of wheat allelochemicals. The paper also highlights the changes in selected soil properties owing to soil incorporation of wheat residue.

Materials and Methods

Residue Collection and Analyses

Whole plant residue of four wheat cultivars (Millat-2011, AARI-2011, Lasani-2008 and Faisalabad-2008) was collected at three different growth stages (tillering (Z-30), anthesis (Z-60) and maturity (Z-90) (Zadoks *et al*., 1974) from plots grown under field conditions at Agronomic Research Area, University of Agriculture Faisalabad, Pakistan (31.25 ˚N, 73.09 ˚E, 184 m above sea level). The residue was chopped with an electric fodder cutter into 2–3 cm pieces and dried under shade to constant moisture content. Total water-soluble phenolics in residue of wheat cultivars collected at different growth stages were quantified as per Swain and Hillis (1959) using Folin-cicalteu's reagent and expressed as gallic acid equivalent. Residue was also analyzed for total carbon and nitrogen following Walkley-Black and kjeldahl digestion methods, respectively (Ryan *et al*., 2001). Measurements were made by repeating the whole procedures thrice, and presented as average value.

Bioassays Study

Canary grass seeds were collected from plants growing in fields with heavy natural infestation. Seeds were cleaned manually to ensure physical purity and surface sterilized with water:bleach solution (10:1) for 15 minutes and then rinsed with distilled water four times. Plastic pots measuring 10 × 26 cm (5 kg capacity) were filled with air-dried and thoroughly mixed field soil. Soil belongs to Lyallpur soil series (Aridisol-fine-silty, mixed, hyperthermic Ustalfic, Haplargid in USDA classification, and Haplic Yermosols in FAO classification scheme; Cheema and Khaliq, 2000). The pH of saturated soil paste and electrical conductivity of the saturation extract were 7.4 and 0.69 dS m^{-1}, respectively. Since it was difficult to sterilize soil in bulk, hence soil was subjected to suicidal germination twice to ensure germination of any viable weed seeds (Khaliq and Matloob, 2012). The residue collected at different growth stages of wheat cultivars was incorporated at the rate of 8 g kg^{-1} soil. The control treatment comprised of soil without residue amendment. After seven days of residue incorporation, twenty seeds of canary grass were sown per pot. These pots were placed in a screen house under natural conditions with a 10/14 h light/dark period at temperature 15˚C±2, and a relative humidity 70% ± 2. The pots were irrigated as and when required to avoid water stress.

Emergence counts were made daily (AOSA, 1990) till emergence became constant. The criterion for emergence was set as >2 mm hypocotyl length as visible to naked eye. Time taken to 50% emergence of seedlings (E_{50}) was calculated according to the modified formulae of Farooq *et al*. (2005):

$$E_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

Where "N" refers to the final number of emerged seeds, and "n_i" and "n_j" are the cumulative number of seeds that emerged by adjacent counts at times "t_i" and "t_j"

where $n_i<N/2<n_j$. Mean emergence time (MET) was calculated as per Ellis and Robert (1981):

$$MET = \frac{\sum Dn}{\sum n}$$

Where "n" denotes the number of seeds, emerged on day "D", and "D" is the number of days counted from the beginning of emergence. Emergence Index (EI) was calculated as described by AOSA (1983):

$$EI = \frac{\text{No.of emerged seeds}}{\text{Days of first count}} + - - - - - - - + \frac{\text{No.of emerged seeds}}{\text{Days of final count}}$$

Seedlings were uprooted carefully at the termination of experiment (6 weeks after sowing) and root and shoot lengths measured with a measuring tape. Roots and shoots from each pot were oven dried at 70°C for 48 h to get dry biomass; total seedling biomass was calculated as the sum of biomass of the root and shoot.

Biochemical Analyses

Total soluble phenolics were determined as described by Randhir and Shetty (2005) and are expressed as gallic acid equivalents. Photosynthetic pigments (Chl. *a* and Chl. *b*) were extracted in 80% ice cold acetone and read out at 663 and 645 nm wavelength in a UV-spectrophotometer (UV-4000, ORI, Germany). These are expressed as mg g^{-1} fresh leaf weight (Arnon, 1949). Soluble proteins were measured using crystalline bovine albumin as a reference (Bradford, 1976). Activity of superoxide dismutase (SOD) was measured as described by Giannopolitis and Ries (1977) at 560 nm. One unit of SOD activity was defined as the amount of enzyme inhibiting the photochemical reduction of NBT by 50% per minute. Catalase (CAT) activity based on the consumption of H_2O_2 was determined using the method of Dhindsa *et al*. (1981). The consumption of H_2O_2 was observed at 240 nm and one unit of CAT was defined as the amount of enzyme required to oxidize 1µ M H_2O_2 min^{-1}. Peroxidase (POX) activity was recorded as described by Egley *et al*. (1983). Increase in absorbance due to guaiacol oxidation was measured at 470 nm. One unit of enzymatic activity was defined as the amount of enzyme required to oxidize 1µ M guaiacol min^{-1}. Lipid peroxidation in leaves of wheat seedling was determined as malondialdehyde (MDA) content following thiobarbituric acid method (Bailly *et al*., 1996). The absorbance of supernatant was read at 532 nm and corrected for non-specific absorbance at 600 nm. The MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm.

Wheat Cultivars Residue Incorporated Soil Analysis

The pH of the wheat-cultivar-residue amended soil was measured by using a digital pH and conductivity meter (HI-9811, Hannah, USA). Soil phenolics were determined with a UV-spectrophotometer (UV-4000, ORI, Germany) as per Box (1983) and are expressed as vanillic acid equivalents. Measurements were made by repeating the whole procedures thrice, and average values are given.

Experimental Design and Statistical Analyses

The experiment was conducted using a completely randomized design with four replications and repeated once. Since the results of two runs of whole experiment were similar, hence the data were pooled for combined analyses. Following Fisher's analysis of variance technique (Steel *et al*., 1997), mean values were separated using least significant difference (LSD) test at $p<0.05$ using the computer statistical program (Statistix 8.1, Analytical software, Statistix; Tallahassee, FL USA, 1985–2003). To ascertain relationship among different variables, correlation computations were also done.

Results

Total Carbon, Nitrogen and Soluble Phenolics in Wheat Residue

Total carbon (C), nitrogen (N) and soluble phenolics in residue of all wheat cultivars were different, and changed with the growth stages of wheat collected (Table 1). Total C and soluble phenolics in all the cultivars increased with advancement in stage of collecting the residue and maximum of these were recorded at maturity. For all wheat cultivars, maximum total N was recorded in residue of anthesis stage, and declined thereafter towards maturity. Such dynamics of total C and N revealed C:N ratio highest at maturity in all the cultivars. Maximum total C (381 mg g^{-1}) and total soluble phenolics (78.88 mg g^{-1} DW) were recorded in residue of wheat cultivar AARI-2011. Similarly, maximum total nitrogen (4.53 mg g^{-1}) was recorded at anthesis in the same cultivar (Table 1). Residue of wheat cultivar Millat-2011 collected at maturity stage depicted highest C:N ratio (103.39) and the corresponding ration for AARI-2011 at this stage was 98.30. Total soluble phenolic in residue of different wheat cultivars ranged from 52–78 mg g^{-1} DW (Table 1).

Effect of Residue Incorporation on Soil Properties

Incorporation of wheat residue at tillering, anthesis and maturity stage changed the selected soil properties as compared to control (Table 2). Total soluble phenolic contents in soil increased at the time of sowing (7 days after residue incorporation) and declined at harvesting time. Upper limit of phenolic contents at sowing and harvesting (24 and 18 mg g^{-1} soil) was recorded for wheat residue of AARI-2011 collected at maturity was incorporated in soil. This shows an increase of 115 and 130% when compared with control at respective stages of determination. Higher organic carbon at the time of sowing and harvesting (0.78 and 1.55%, and 0.72 and 1.80%)

Table 1: Chemical properties of wheat residue collected at different growth stages

	Millat-2011			AARI-2011			Lasani-2008			Faisalabad-2008		
	Tillering	Anthesis	Maturity	Tillering	Anthesis	Maturity	Tillering	Anthesis	Maturity	Tillering	Anthesis	Maturity
Total C (mg g^{-1})	131.54± 0.70	326.46± 0.46	376.34± 1.28	142.54± 0.59	342.65± 1.67	381.44± 0.87	122.33± 0.96	325.57± 0.85	334.77± 1.23	122.43± 1.63	322.35± 1.42	345.68± 1.30
Total N (mg g^{-1})	2.29± 0.05	4.09± 0.06	3.64± 0.04	2.47± 0.05	4.53± 0.05	3.88± 0.23	2.32± 0.05	4.40± 0.06	3.67± 0.04	2.31± 0.05	4.01± 0.07	3.55± 0.02
C:N	57.44	79.82	103.39	57.71	75.64	98.30	52.73	73.99	91.22	53.00	80.38	97.37
Total soluble phenol (mg g^{-1} D.W)	55.45± 2.42	61.83± 3.91	71.08± 3.49	57.89± 1.76	60.18± 2.36	78.88± 2.57	52.32± 0.87	60.14± 1.51	70.91± 1.84	54.68± 1.22	58.70± 1.26	68.41± 2.15

D.W: dry weight; ± S.E

Table 2: Influence of incorporation of wheat residue collected at different growth stages on total soluble phenolic contents, pH, organic carbon and available nitrogen in soil

Growth stage	Millat-2011		AARI-2011		Lasani-2008		Faisalabad-2008	
	*At sowing	**At harvesting	At sowing	At harvesting	At sowing	At harvesting	At sowing	At harvesting
	Total soluble phenolics (mg g^{-1} soil)							
Control	11.24±0.85	7.99±1.29	11.24±0.85	7.99±1.29	11.24±0.85	7.99±1.29	11.24±0.85	7.99±1.29
Tillering	14.01±0.84	10.97±1.16	15.91±0.95	14.91±0.89	14.79±0.89	10.79±0.85	13.68±0.82	9.71±1.04
Anthesis	17.02±1.02	12.01±0.84	21.70±1.30	15.76±1.30	15.24±0.91	13.76±1.22	14.57±0.87	13.35±1.11
Maturity	19.84±0.75	16.74±0.85	24.20±1.49	18.40±1.53	18.02±1.08	15.24±0.91	16.24±0.97	14.90±1.66
	pH							
Control	7.4±0.04	7.2±0.06	7.4±0.04	7.2±0.06	7.4±0.04	7.2±0.06	7.4±0.04	7.2±0.06
Tillering	7.4±0.05	7.6±0.06	7.5±0.01	7.6±0.05	7.5±0.05	7.7±0.06	7.4±0.01	7.6±0.03
Anthesis	7.5±0.05	7.7±0.07	7.6±0.07	7.8±0.05	7.6±0.07	7.8±0.01	7.5±0.06	7.7±0.03
Maturity	7.6±0.08	7.7±0.02	7.7±0.03	7.8±0.01	7.6±0.02	7.8±0.05	7.6±0.04	7.8±0.00
	Organic carbon (%)							
Control	0.38±0.15	0.14±0.06	0.38±0.15	0.14±0.06	0.38±0.15	0.14±0.06	0.38±0.15	0.14±0.06
Tillering	0.55±0.09	1.40±0.08	0.59±0.12	1.55±0.07	0.42±0.06	1.44±0.08	0.37±0.09	1.34±0.10
Anthesis	0.49±0.08	1.52±0.13	0.78±0.11	1.55±0.11	0.65±0.15	1.48±0.07	0.60±0.11	1.39±0.07
Maturity	0.66±0.12	1.76±0.08	0.72±0.10	1.80±0.11	0.95±0.07	1.71±0.11	0.76±0.13	1.55±0.09
	Available nitrogen (kg ha^{-1})							
Control	89.23±1.78	51.84±1.65	89.23±1.78	51.84±1.65	89.23±1.78	51.84±1.65	89.23±1.78	51.84±1.65
Tillering	101.66±1.58	129.95±1.29	122.08±1.41	144.26±2.01	112.06±1.27	134.36±1.97	104.79±1.89	130.09±1.09
Anthesis	127.39±0.91	152.05±1.16	139.33±1.45	161.96±1.63	125.11±2.30	152.63±1.72	117.61±1.93	150.36±2.12
Maturity	141.89±1.49	180.85±2.10	144.48±1.86	183.72±2.38	136.60±1.48	176.08±0.87	131.89±1.03	169.32±1.92

± S.E, * Soil samples were collected after 7 days of wheat residue incorporation at the time of sowing, **Soil samples were collected at the time of harvesting; Each value is average of four replicates

and available nitrogen (139 and 161 kg ha^{-1}, and 144 and 183 kg ha^{-1}) was recorded for soil incorporated residue of cultivar AARI-2011 collected at anthesis and maturity stage (Table 2). The pH of the residue-amended soil was higher at the time of sowing (7.5±0.2) and harvesting (7.6±0.2) as compared to control (7.2).

Effects on Seedling Emergence and Growth

Soil incorporation of wheat residue of four wheat cultivars (Millat-2011, AARI-2011, Lasani-2008 and Faisalabad-2008) collected at different growth stages significantly ($p\leq0.05$) affected the emergence of canary grass (Table 3). Interactive effect between wheat cultivars and stages of residue collection was non-significant ($p\leq0.05$) for most attributes except MET, whereas the influence of wheat cultivars was significant ($p\leq0.05$) for time taken to 50% emergence only (Table 3). Nevertheless, stage of collection of residue has pronounced effect on emergence dynamics of canary grass. Time to start emergence and 50% emergence was significantly ($p\leq0.05$) delayed by 2 to 3 days as compared to control. The EI of canary grass was lower by 15 and 30% as compared to control, with soil incorporation of crop residues at anthesis and maturity (Table 3). Significant ($p\leq0.05$) cultivar × stages of residue collection interaction showed that MET did not vary at tillering stage for all wheat cultivars. Nevertheless, such differences were pronounced for residue at anthesis and maturity stage and effects were cultivar specific (Fig. 1). Maximum delay in MET of canary grass was observed for soil incorporation of wheat cultivar AARI-2011 residues at maturity stage however, MET was similar ($p\leq0.05$) for incorporation of residues at anthesis of the same cultivar and with cv. Lasani-2008 at maturity stage. Soil incorporation of wheat residue at anthesis and maturity stage significantly ($p\leq0.05$) suppressed final emergence percentage of canary grass by 13 and 31% over control (Table 3). This effect was statistically non-significant, but soil incorporation of wheat residue at tillering had stimulatory effect on emergence attributes of canary grass as compared to control (Table 3).

A significant ($p\leq0.05$) interactive influence of wheat cultivars and stage of residue collection was recorded on seedling growth of canary grass for all the traits (Fig. 2).

Table 3: Influence of soil incorporation of wheat residue collected at different growth stages on emergence dynamics of canary grass

Growth stage	Millat-2011	AARI-2011	Lasani-2008	Faisalabad-2008	Mean
	Time to start emergence (days)				
Control	6.25$^{n.s}$	6.25	6.25	6.25	6.25 B
Tillering	6.50	6.25	6.50	6.25	6.38 B
Anthesis	7.75	8.00	8.25	8.00	8.00 A
Maturity	7.75	8.75	8.75	8.50	8.44 A
Mean	7.06	7.31	7.44	7.25	
LSD *p≤0.05*	C=n.s, G.S=0.801, C×G.S=n.s				
	Time taken to 50% emergence (days)				
Control	9.23$^{n.s}$	9.23	9.23	9.23	9.23 B
Tillering	9.31	9.51	9.74	9.36	9.48 B
Anthesis	10.81	11.79	12.54	12.57	11.93 A
Maturity	10.55	12.00	11.25	12.17	11.49 A
Mean	9.97 B	10.63 A	10.69 A	10.83 A	
LSD *p≤0.05*	C=0.588, G.S=0.588, C×G.S=n.s				
	Emergence index				
Control	7.03$^{n.s}$	7.03	7.03	7.03	7.03 A
Tillering	7.53	7.56	7.57	7.64	7.58 A
Anthesis	5.38	5.65	5.87	7.15	6.01 B
Maturity	5.30	4.52	4.51	5.43	4.94 C
Mean	6.31	6.19	6.24	6.82	
LSD *p≤0.05*	C=n.s, G.S=0.699, C×G.S=n.s				
	Final emergence (%)				
Control	98.75$^{n.s}$	98.75	98.75	98.75	98.75 A
Tillering	100.00	98.75	100.00	98.75	99.38 A
Anthesis	80.00	85.00	85.00	91.25	85.31 B
Maturity	75.00	67.50	71.25	77.50	72.81 C
Mean	88.44	87.50	88.75	91.56	
LSD *p≤0.05*	C=n.s, G.S=6.882, C×G.S=n.s				

C= wheat cultivars, G.S= growth stages, C×G.S= wheat cultivars × growth stages, n.s= non-significant. Each number is average of four replicates; Main effect means not sharing a letter in common differ significantly at 5% probability level by LSD test

Maximum reduced shoot length of canary grass was observed for residue at maturity of cv. AARI-2011 and Lasani-2008 (Fig. 2a) and this, was statistically similar with little canary grass shoot length recorded for Millat-2011 residue incorporated at the same stage. Shoot length of canary grass was enhanced (15–24 %), where residue of both Millat-2011 and Faisalabad-2008 collected at tillering stage was soil incorporated. Incorporation of residue at tillering of wheat cultivars Millat-2011, AARI-2011 and Faisalabad-2008 enhanced root length of canary grass by 17, 16 and 29% over control (Fig. 2b). However, at anthesis and maturity stage of AARI-2011 and Lasani-2008 recorded highest suppression (34–52% and 28–54%) of root length of canary grass over control. Seedling fresh and dry biomass varied significantly ($p\leq0.05$) among wheat cultivars as a function of stage of residue collection. Incorporation of residue at tillering of Millat-2011 and Faisalabad-2008 stimulated the fresh biomass of canary grass seedling over control (Fig. 2c) as against AARI-2011 and Lasani-2008, which did not enhance fresh biomass of canary grass over control. Seedling biomass was suppressed to highest extent with soil incorporation of residue of all wheat cultivars collected at maturity, and the differences among cultivars were non-significant. Seedling dry biomass of canary grass

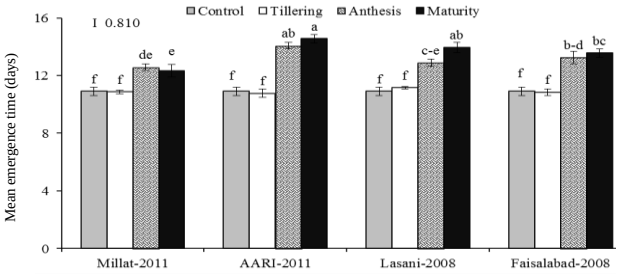

Fig. 1: Influence of soil incorporation of wheat residue of different growth stages on mean emergence time of canary grass

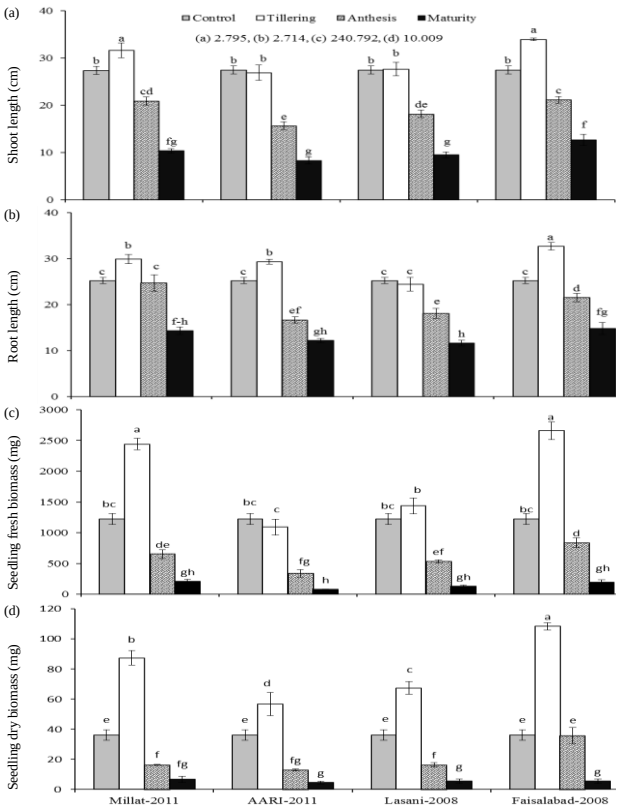

Fig. 2: Influence of soil incorporation of wheat residue collected at different growth stages on seedling growth of canary grass

was significantly ($p\leq0.05$) improved over control where residue was incorporated at tillering of all wheat cultivars was incorporated (Fig. 2d). On the contrary, the residue-maturity had a suppressive effect on seedling dry biomass of canary grass so that Millat-2011, AARI-2011, Lasani-2008 and Faisalabad-2008 recorded 82, 88, 85 and 86% reduction in it as compared to control; the differences among these being non-significant. Nevertheless, incorporation of residue-maturity stage resulted in statistically similar suppression of canary grass irrespective of wheat cultivars.

Biochemical Attributes

Chlorophyll and soluble phenolic contents: Leaf chlorophyll *"a"* (Chl *a*) content of canary grass grown in soil amended with wheat residue-anthesis and -maturity stage was reduced by 52 and 71%, respectively over control. The corresponding decreased in chlorophyll *"b"* (Chl *b*) was 36 and 54% (Table 4). Soil incorporation of residue collected at tillering stage improved Chl *"a"* and *"b"* content by 20 and 13% over control. There was an interactive and significant ($p \leq 0.05$) effect of wheat cultivars and the stage at which residue was collected on total chlorophyll content of canary grass seedling. Total chlorophyll contents were significantly ($p \leq 0.05$) higher than control where wheat residue (Millat-2011 and Faisalabad-2008) collected at tillering stage was soil incorporated. Maximum reduction in total chlorophyll content (71%) of canary grass seedling was observed where residue was incorporated at maturity stage of cv. AARI-2011 was incorporated (Fig. 3). Phenolic content in leaves of canary grass varied significantly ($p \leq 0.05$) under the influence of residue of different growth stages, and were increased by 30, 22 and 13% with the soil incorporation of residue at tillering, anthesis and maturity stages as compared to control (Table 4).

Lipid Peroxidation and Activity of Enzymatic Antioxidants

The MDA contents of canary grass leaves were increased by 85 and 142% over control when grown in soil incorporated with residue at anthesis and maturity stages of wheat. Incorporation of residue at tillering stage had no effect on MDA contents. Significantly ($p \leq 0.05$) higher MDA contents were observed for canary grass seedlings grown in pots incorporated with residue of AARI-2011 and Lasani-2008 cultivars. MDA content in canary grass for different cultivars of wheat was in order of AARI-2011> Lasani-2008> Millat-2011>Faisalabad-2008 (Table 5). Wheat cultivars and the stage of collection of residue have a significant effect on superoxide dismutase (SOD), catalase (CAT) and soluble proteins (Fig. 4) in canary grass seedling. Minimum increase in activity of SOD was observed over control with incorporation of crop residue at maturity stage of each wheat cultivars as compared with that of tillering. Amongst all stages, residue incorporation at maturity was least effective in increasing the SOD activity in canary grass. SOD activity was significantly ($p \leq 0.05$) highest for residue at tillering in each wheat cultivars (Fig. 4a). Highest SOD activity was noticed in canary grass seedling amended with residues at tillering of wheat cultivar Lasani-2008. Activity of CAT in canary grass seedling either became higher (Millat-2011), remained similar (AARI-2011, Lasani-2008) or was lower (Faisalabad-2008) than control when incorporated with residue at tillering of wheat cultivars (Fig. 4b). Interaction of wheat cultivars with stages of residue collection was non-significant for POX activity. However, POX activity in canary grass seedling was significantly ($p \leq 0.05$) affected by wheat residue of different growth stages. Maximum POX activity was noticed for wheat residue at tillering and was similar with control. However, significant ($p \leq 0.05$) decline in POX activity of canary grass seedling was found when amended with residue at maturity stage and was at par for

Table 4: Influence of soil incorporation of wheat residue collected at different growth stages on chlorophyll "a", chlorophyll "b" and total phenolic contents in canary grass

Growth stage	Millat-2011	AARI-2011	Lasani-2008	Faisalabad-2008	Mean
	Chlorophyll "a" (mg g^{-1} FW)				
Control	12.23	12.23	12.23	12.23	12.23 B
Tillering	17.74	13.56	13.12	14.21	14.66 A
Anthesis	6.37	4.85	6.23	5.92	5.84 C
Maturity	3.67	2.29	2.72	5.03	3.59 D
Mean	10.00	8.23	8.58	9.35	
LSD *p≤0.05*	C=n.s, G.S=1.353, C×G.S=n.s				
	Chlorophyll "b" (mg g^{-1} FW)				
Control	5.86	5.86	5.86	5.86	5.86 B
Tillering	7.26	6.05	6.16	7.10	6.64 A
Anthesis	4.24	3.30	3.94	3.51	3.75 C
Maturity	2.90	1.99	2.98	2.78	2.66 D
Mean	5.06	4.30	4.73	4.81	
LSD *p≤0.05*	C=n.s, G.S=0.501, C×G.S=n.s				
	Total soluble phenolic (mg g^{-1} FW)				
Control	43.79	43.79	43.79	43.79	43.79 C
Tillering	64.78	57.75	51.06	54.37	56.99 A
Anthesis	57.19	56.60	48.11	53.01	53.73 A
Maturity	48.39	54.93	44.90	50.13	49.59 B
Mean	53.54 A	53.27 A	46.97 B	50.33 AB	
LSD *p≤0.05*	C=3.989, G.S=3.989, C×G.S=n.s				

C= wheat cultivars, G.S= growth stages, C×G.S= wheat cultivars × growth stages, n.s= non-significant. Each number is average of four replicates; Main effect means not sharing a letter in common differ significantly at 5% probability level by LSD test

Table 5: Influence of soil incorporation of wheat residue collected at different growth stages on POX activity and MDA content in canary grass

Growth stage	Millat-2011	AARI-2011	Lasani-2008	Faisalabad-2008	Mean
	MDA (nmol g^{-1} FW)				
Control	0.68	0.68	0.68	0.68	0.68 C
Tillering	0.76	0.98	0.87	0.76	0.84 C
Anthesis	1.15	1.35	1.32	1.21	1.26 B
Maturity	1.40	2.12	1.70	1.38	1.65 A
Mean	1.00 B	1.28 A	1.14 AB	1.01 C	
LSD *p≤0.05*	C=0.223, G.S=0.223, C×G.S=n.s				
	POX (µmol min^{-1} g^{-1} protein)				
Control	9.77	9.77	9.77	9.77	9.77 AB
Tillering	12.46	12.10	12.76	12.37	12.42 A
Anthesis	8.04	8.55	7.62	10.66	8.72 BC
Maturity	5.45	6.99	6.19	6.95	6.39 C
Mean	8.93	9.35	9.09	9.94	
LSD *p≤0.05*	C=n.s, G.S=2.701, C×G.S=n.s				

C= wheat cultivars, G.S= growth stages, C×G.S= wheat cultivars × growth stages, n.s= non-significant Each number is average of four replicates; Main effect means not sharing a letter in common differ significantly at 5% probability level by LSD test

residue at anthesis (Table 5). Soil incorporation of residue at tillering of Millat-2011 significantly ($p \leq 0.05$) improved soluble protein contents and of other wheat cultivars was not significantly ($p \leq 0.05$) different from control when compared for this particular stage of residue collection. For incorporation of residue at anthesis stage of wheat cv. Faisalabad-2008, minimum soluble protein in canary grass seedling was observed. Soluble protein contents of canary grass decreased by 51% where residue at maturity of wheat cultivar AARI-2011 was soil incorporated (Fig. 4c).

Correlation between Variables

Regression analyses showed that no correlation existed between final emergence percentage (FEP) and seedling dry biomass of canary grass grown in pots amended with residue at tillering stage of wheat. These traits were positively associated with wheat residue at anthesis and maturity stage. Leaf chlorophyll contents of canary grass manifested a strong positive correlation with seedling fresh biomass and found true for all experimental treatments except for treatment incorporated with residue at tillering stage of Lasani-2008 cultivar. Correlation between total chlorophyll and soluble phenolic contents of canary grass was negative where residue of wheat cultivars collected at different growth stages was soil incorporated (Table 6).

There was a strong and positive relationship between SOD activity and seedling dry biomass of canary grass under the influence of residue incorporation collected at tillering stage of all four wheat cultivars. However, such an association was non-significant for all four wheat cultivars when compared for anthesis and maturity stage residue (Table 6). Activities of POX and CAT were positively correlated with seedling dry biomass of canary grass for pots amended with residue of different growth stages of all wheat cultivars. Likewise, protein contents were positively correlated with seedling dry biomass of canary grass under the influence of anthesis and maturity stage residue incorporation of all wheat cultivars. Nevertheless, such a relationship was non-significant for anthesis stage residue of Faisalabad-2008. However; for tillering stage residue, such correlation was significant only for Millat-2011(Table 6).

Table 6: Correlation analyses showing strength of association between different variables

X-variable	Y-variable	Cultivars	Growth stages		
			Tillering	Anthesis	Maturity
FEP	SDW	Millat-2011	0.426$^{n.s}$	0.691*	0.856**
		AARI-2011	0.149$^{n.s}$	0.714*	0.803**
		Lasani-2008	0.503$^{n.s}$	0.661*	0.739*
		Faisalabad-2008	0.101$^{n.s}$	0.642*	0.796**
Chlorophyll	SFW	Millat-2011	0.734*	0.961***	0.995***
		AARI-2011	0.695*	0.989***	0.993***
		Lasani-2008	0.461$^{n.s}$	0.979***	0.998***
		Faisalabad-2008	0.842**	0.847**	0.993***
Chlorophyll	Total soluble phenolics	Millat-2011	-0.605*	-0.804**	-0.741*
		AARI-2011	-0.628*	-0.944***	-0.921***
		Lasani-2008	-0.918***	-0.818**	-0.158$^{n.s}$
		Faisalabad-2008	-0.654*	-0.929***	-0.746*
SOD	SDW	Millat-2011	0.931***	-0.166$^{n.s}$	-0.164$^{n.s}$
		AARI-2011	0.838**	-0.305$^{n.s}$	-0.308$^{n.s}$
		Lasani-2008	0.837**	-0.349$^{n.s}$	-0.232$^{n.s}$
		Faisalabad-2008	0.825**	0.210$^{n.s}$	-0.119$^{n.s}$
POX	SDW	Millat-2011	0.738*	0.856**	0.866**
		AARI-2011	0.862**	0.774**	0.762**
		Lasani-2008	0.829**	0.874**	0.889**
		Faisalabad-2008	0.708**	0.931***	0.838**
CAT	SDW	Millat-2011	0.902***	0.790**	0.850**
		AARI-2011	0.702*	0.929***	0.965***
		Lasani-2008	0.817**	0.907***	0.871**
		Faisalabad-2008	-0.686*	0.418$^{n.s}$	0.938***
Protein	SDW	Millat-2011	0.963***	0.909***	0.900***
		AARI-2011	-0.210$^{n.s}$	0.923***	0.979***
		Lasani-2008	0.252$^{n.s}$	0.933***	0.935***
		Faisalabad-2008	0.426$^{n.s}$	0.339$^{n.s}$	0.965***

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, n.s= non-significant

Discussion

The differential allelopathic effect exerted by wheat residue on canary grass seedling in present study can be attributed to differences in type and concentration of allelochemicals present in four wheat cultivars that varied at different growth stages. The degree of inhibition and stimulation also strongly correspond to concentration of allelochemicals released into the soil from residue of wheat cultivars collected at different growth stages (Kuk *et al*., 2001) (Table 3–5; Figs. 2–4). Suppression of the emergence with incorporation of wheat residue may be due to the exposure of seed to released allelochemicals from residue, which might altered the biochemical and physiological processes of cell membrane ultra-structures, membrane permeability and integrity, synthesis of certain compounds and enzymatic activity during germination (Gniazdowska and Bogatek, 2005). Reduced speed of emergence of canary grass was an indication of the inhibitory effect of allelochemicals released by decomposing wheat residue over time course. Seedling growth of canary grass was significantly suppressed by interactive effect of wheat cultivars × stage of collection of residue. Suppression in seedling growth of canary grass might be due to the inhibitory effect of allelochemicals either by creating the physiological drought, reduction of cell division and elongation, or by reduced stimulatory growth (Al-Wakeel *et al*., 2007). These allelochemicals may cause alteration in cell membrane structure and permeability which results in several other cross-stress responses due to ROS damage (Khaliq *et al*., 2012) and lipid peroxidation (Zeng *et al*., 2001). The increased activity of antioxidant enzymes as observed in present study confirm this (Table 5; Fig. 4). Oxidative stress can alter the membrane permeability and cause fluxes across plasma membrane causing enzyme activation, oxidative stress and root uptake. Plants overcome these changes by the activation of antioxidant system and regulating the enzyme activity (Bogatek and

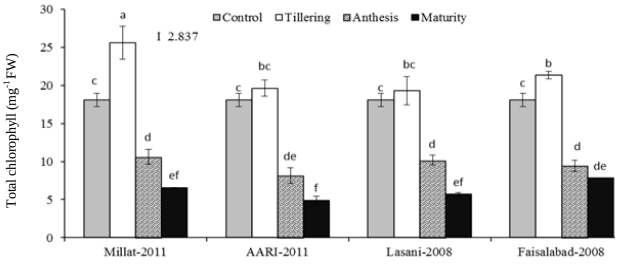

Fig. 3: Influence of soil incorporation of wheat residue collected at different growth stages on total chlorophyll contents of canary grass

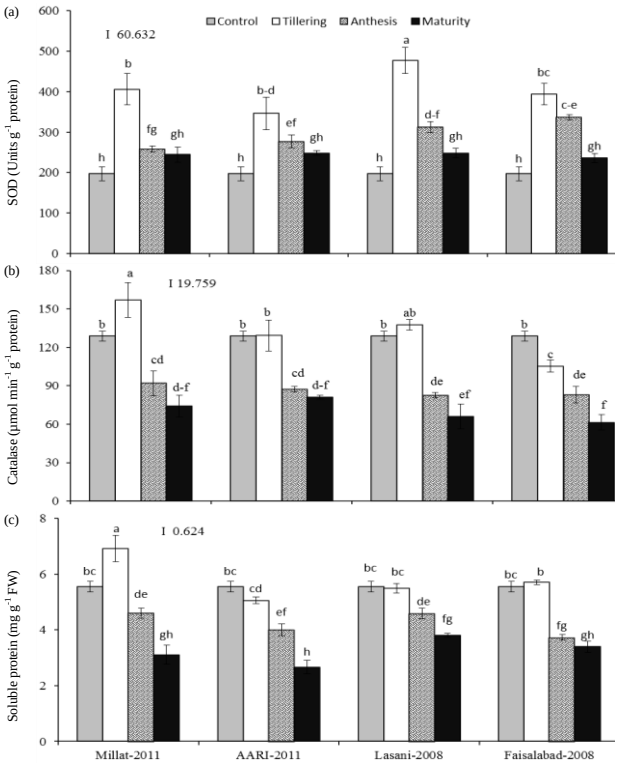

Fig. 4: Influence of soil incorporation of wheat residue of different growth stages on antioxidant activities of canary grass

Gniazdowska, 2007).

Decrease in chlorophyll contents in the leaves of canary grass with soil incorporation of wheat residue of all wheat cultivars collected at anthesis and maturity may be due to the activity of released allelochemical compounds. Decline in chlorophyll contents was highly associated with production of phenolic compounds (Table 5). Recently, Sumbele *et al.* (2012) reported with 49 plant species of Greece and Australia that photosynthetic capacity was negatively associated with leaf phenolic contents. Wheat allelochemical compounds might have inhibited chlorophyll content in canary grass seedlings either by interfering with the biosynthesis of photosynthetic pigments or by enhancing their degradation by inducing oxidative stress and generation of reactive oxygen species or both. This was substantially supported by significantly lower chlorophyll contents and increased activities of enzymatic antioxidants observed during in present study (Table 4, Fig. 3).

The antioxidant enzymes are often activated under stress conditions. SOD activity of canary grass increased when grown in soil amended with wheat residue of tillering stage that was helpful in reducing the adverse effects of wheat allelochemicals originating from superoxidase radicles (O_2^-). The SOD is a metalo enzyme and involved in the dismutation of superoxide radicles (O_2^-) (Apel and Hert, 2004). The stimulation of SOD activity removes the O_2^- contents and produces H_2O_2 and O_2. On the other hand, increased activity of POX and CAT is helpful to scavenge H_2O_2 and O_2 into H_2O and O_2. Under lower concentration of phytotoxic compounds, free radicals are easily alleviated by scavengers due to less stress conditions (Zhang *et al.*, 2010). In addition, the MDA contents did not change much over control and this may be due to higher SOD activity which reduced O_2^- contents. Zhang *et al.* (2010) found that higher SOD activity reduced the lipid peroxidation in membranes. The SOD, POX and CAT activities of canary grass were; however, lower when grown in soil amended with wheat residue at anthesis and maturity stages. It may be because of higher concentration of allelochemicals in wheat residue collected at anthesis and maturity as evident from higher phenolic contents in such residue (Table 1). Huang *et al.* (2010) also reported that increasing concentration of allelochemicals inhibited the activity of SOD. The allelochemicals induced oxidative stress in the target tissues and hampered their antioxidant mechanism (Aenavoli *et al.*, 2006). It seems that wheat phytotoxic compounds cause oxidative stress by stimulating lipid peroxidation of membrane in stressed canary grass seedlings as indicated by higher MDA contents in such seedlings.

Inhibition of host plant's growth by incorporation of residue depends on residue quality, degree of decomposition, nutrient status of residue and microbial activity (Rice, 1984; Inderjit *et al.*, 1996). Residue of all wheat cultivars collected at different growth stages significantly varied for their total carbon, nitrogen, and carbon/nitrogen ratio. Residue incorporation at anthesis and maturity stage of wheat cultivars had higher carbon and nitrogen contents as compared to tillering stage. Maximum total carbon (381 and 376 mg g^{-1}) was recorded in residue of AARI-2011 (anthesis) and Millat-2011 (maturity) and highest nitrogen (4.53 and 4.40 mg g^{-1}) contents were determined at anthesis for AARI-2011 and Lasani-2008. However, residue collected at tillering stage decomposed rapidly as compared to anthesis and/or maturity stages. Nature of the incorporated residue affects microbial activity, and cycling and decomposition of carbon and other nutrient elements (Sajjad *et al.*, 2002). Soil microorganisms decompose these compounds (Inderjit, 2005; Jilani *et al.*, 2008) and improve their activity by

returning the straw of cover crops into the soil using organic carbon as an energy source (Hai-Ming *et al*., 2014). The addition of plant residue in the soil enhances the microbial activity, and population (Ruiyu *et al*., 2007), which utilize the nitrogen present in the soil consequently causing temporary nitrogen deficiency (Harper, 1977). It is likely that the observed inhibitory effect of soil incorporation of wheat residue are due to nitrogen depletion by rapidly growing populations of microbes rather than release of phytotoxic compounds during decomposition. However, in present study available nitrogen was higher in residue-amended soil as compared to unamended soil. Sodaeizadeh *et al*. (2010) concluded that the negative impact of *Peganum harmala* L. amended soil on growth of *Avena fatua* L. and *Convolvulus arvensis* L. are not due to nitrogen immobilization. Bonanomi *et al*. (2011) also reported the inhibitory effects of plant litter on *Lepdium sativum* L. owing to phytotoxicity, rather than N mobilization. Furthermore, the effect of residue decomposition on the soil pH varied during the course of study (Table 2). This may be due to the differences in residue composition and release of carbon and nitrogen compounds. Soil incorporation of wheat residue of different growth stages increased the nitrogen contents and pH of the soil over time (Table 2). Numerous studies have reported that the pH dependent mineralization of carbon and nitrogen (Marschner and Kalbitz, 2003; Kemmitt *et al*., 2006). Lower pH often reduces the mineralization by suppressing the activity and survival of microorganisms, and decreases the solubility of dissolved organic compounds (Butterly *et al*., 2013).

Majority of allelochemicals produced by wheat are phenolic acids, hydroximic acid, and short and long chain fatty acids (Wu *et al*., 2001; Ma, 2005). These compounds are allelopathic for weeds as well as other crop plants (Wu *et al*., 2001). Numerous studies reported that allelopathic potential varied among cultivars of same species (Zuo *et al*., 2007; Anjum and Bajwa, 2010), among plant parts (Qasem and Foy, 2001), plant age (Wu *et al*., 2000) and environmental conditions (Rice, 1984). Our results revealed that residue of all four wheat cultivars contained different concentration of phenolic compounds that increased with advancement in stage of their growth (Table 1). Soil amended with wheat residue at anthesis and maturity stages depicted higher concentration of allelochemicals as compared to soil amended with residue at tillering stage (Table 2), obviously leading to higher allelopathic for canary grass.

Conclusion

The present study concludes that decomposing wheat residue showed inhibitory effect against canary grass that varied among wheat cultivars and the growth stage at which it was collected. Phenolic contents increased with stage of development of wheat, and residue of wheat cultivars AARI-2011 and Lasani-2008 collected and soil incorporated at anthesis and maturity were more phytotoxic than of Millat-2011 and Faisalabad-2008. Scavenging of phytotoxic effects of phelnolic compounds were accomplished by enhancing the activity of SOD, calatalse, and POX.

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