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Article

Allelopathic Effect of Some Associated Weeds of Wheat on Germinability and Biomass Production of Wheat Seedlings

Efeito Alelopático de Algumas Plantas Daninhas do Trigo Associadas na Germinabilidade e Produção de Biomassa de Plântulas do Trigo

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ABSTRACT - Weeds associated with crops may impose their phytotoxic effects on crop plants through the release of their allelochemicals and hence seriously reduce crop productivity. The present study was conducted to investigate the allelopathic effect of water soluble phenolics of weeds associated with wheat crop (*Vicia sativa*, *Trigonella polycerata*, *Lathyrus aphaca*, *Medicago polymorpha*, *Melilotus indica*) on germinability and biomass production of wheat seedlings by using their water extracts at 2.5% (w/v) and 5% (w/v) concentrations and residues of the same weeds with 0, 15 and 30 d decomposition periods at 2% (w/w) concentration. The results showed that the water extracts of *M. indica* and *V. sativa* at 5% concentration imposed the most inhibitory effect on energy of germination (81%) and prolonged time to 50% germination (226%), respectively; by contrast, *T. polycerata* and *M. indica* extracts at the same concentration inhibited shoot (8%) and root fresh biomass (64%). However, shoot dry biomass was exalted by the weed extracts at both concentrations with maximum increase (29%) caused by the *M. indica* extract at 2.5% concentration; however, root dry biomass (46%) and root/shoot ratio (51%) were decreased by *M. polymorpha* and *M. indica* extracts at 5% concentration, respectively. Residues of *L. aphaca* and *M. polymorpha* with a 30 d decomposition period proved the most toxic regarding energy of emergence (85%) and time to 50% emergence (138%), respectively; while, shoot fresh (41%) and dry biomass (26%) production were hindered mostly by *M. indica* and *M. polymorpha* residues with a 30 d decomposition period. There was the highest decrease in root fresh (64%) and dry biomass (64%), and root/shoot ratio (64%) when treatment was performed with *V. sativa* residues without decomposition. The results show that leachates and residues of weeds inhibit wheat germinability and biomass production through release of allelochemicals, and they are a threat to profitable crop production.

Keywords: wheat, weed allelopathy, water extracts, residues, germination, biomass production

*RESUMO - Plantas daninhas associadas a culturas agrícolas podem impor efeitos fitotóxicos sobre as plantas cultivadas através da liberação de aleloquímicos, reduzindo consideravelmente a produtividade das culturas. O objetivo do presente estudo foi investigar o efeito alelopático de fenóis hidrossolúveis de plantas daninhas associadas ao trigo (**Vicia sativa**, **Trigonella polycerata**, **Lathyrus aphaca**, **Medicago polymorpha** e **Melilotus indica**) sobre a germinabilidade e a produção de biomassa de plântulas de trigo, utilizando os extratos aquosos dessas plantas daninhas nas concentrações de 2,5% (p/v) e 5% (p/v) e dos resíduos delas com períodos de decomposição de 0, 15 e 30 d, na concentração de 2% (p/p). Os*

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resultados mostraram que os extratos aquosos de ***M. indica*** *e* ***V. sativa*** *na concentração de 5% exerceram maior efeito inibidor sobre a energia de germinação (81%) e o tempo para 50% de germinação (226%), respectivamente, enquanto os extratos de* ***T. polycerata*** *e* ***M. indica****, na mesma concentração, inibiram a biomassa fresca da parte aérea (8%) e da raiz (64%). No entanto, foi observado aumento da biomassa seca da parte aérea por conta dos extratos das plantas daninhas em ambas as concentrações, com aumento máximo (29%) causado pelo extrato de* ***M. indica*** *na concentração de 2,5%; contudo, a biomassa seca da raiz e a razão raiz/parte aérea tiveram redução de 46% e 51%, respectivamente, devido aos extratos de* ***M. polymorpha*** *e* ***M. indica****. Os resíduos de* ***L. aphaca*** *e* ***M. polymorpha*** *com 30 dias de decomposição demonstraram o maior nível de toxicidade em relação à energia de emergência (85%) e ao tempo de 50% de emergência (138%), respectivamente. Já a produção de biomassa fresca (41%) e de biomassa seca (26%) foi dificultada principalmente pelos resíduos de* ***M. indica*** *e* ***M. polymorpha*** *com 30 d de decomposição. Observou-se a maior redução na biomassa fresca (64%) e na biomassa seca (64%) da parte aérea, bem como na razão raiz/parte aérea (64%), quando o tratamento foi realizado com resíduos de* ***V. sativa*** *sem decomposição. Os resultados mostram que lixiviados e resíduos de plantas daninhas inibem a germinabilidade do trigo e a produção de biomassa através da liberação de aleloquímicos e são uma ameaça para a produção rentável de culturas agrícolas.*

Palavras-chave: trigo, alelopatia de plantas daninhas, extratos aquosos, resíduos, germinação, produção de biomassa.

INTRODUCTION

A large number of weeds are found associated with various crops and are a major threat to crop production in many cropping systems. It has been estimated that associated weeds of wheat may cause yield reductions up to 25-30% (Chaudhary et al., 2008; Marwat et al., 2008) depending upon the duration of association and density of weeds, types of weeds and management practices employed. Weeds impose inhibitory as well as stimulatory allelopathic influences on crop plants through allelopathic interactions. A large number of allelopathic weeds have been documented in the literature; they affect crop plants right from emergence to maturity and cause considerable economic losses (Jalageri et al., 2010; Tanveer et al., 2010; Mohadesi et al., 2011; Muzaffar et al., 2012; Zohaib et al., 2014a).

Allelopathy is any process whereby secondary metabolites produced by plants, microorganisms, viruses, and fungi influence the growth and development of agricultural and biological systems, including positive and negative effects (Torres et al., 1996). Actually, allelochemicals are secondary metabolites released from plants and affect the germination and growth processes of plants in their vicinity (Rehman, 2005; Zhang et al., 2015). Plants have a diverse mode of release of allelochemicals such as residue decomposition, leaching from leaf litter and other plant parts, volatilization and root exudation (Narwal et al., 2005). However, in field conditions, the release of allelochemicals mostly takes place through decomposition of plant residues (Singh et al., 2001). Seed germination, plant growth and biomass production are hindered by numerous allelochemicals through disruption of a variety of physiological functions taking place within plant body (Zohaib et al., 2016). The plant functions of prime importance which are affected by allelochemicals include photosynthesis, respiration, mitosis, cell elongation and enlargement, metabolic activities, synthesis of proteins and amino acids, and enzyme activities (Shao-Lin et al., 2004). It has been observed that a differential allelopathic effect is imposed by different plant species on recipient plants (Hamayun et al., 2005). Similarly, allelochemicals behave differently in exerting allelopathic influence on recipient plants, depending upon their concentration. Generally, higher concentrations of allelochemicals are inhibitory in their action while, on other hand, lower concentrations are associated with stimulation of germination and growth of plants coming in contact with them (Hossain and Alam, 2010). Furthermore, the allelopathic effect of decomposing residues also varies with variation in the length of their decomposition (Teerarak et al., 2010).

Many weeds are found in association with wheat (*Triticum aestivum* L.) crops; however, *Vicia sativa* L. (common vetch), *Trigonella polycerata* L. (trefoil), *Lathyrus aphaca* L. (yellow vetchling), *Medicago polymorpha* L. (bur clover) and *Melilotus indica* L. (Indian sweet clover) are weeds most commonly occurring in many temperate and tropical parts of the world including Australia, Africa, Europe, the United States and many Asian countries including Pakistan and India. Although these are found in association with almost all winter season crops, they are commonly associated with wheat (Ahmad and Shaikh, 2003). These are leguminous weeds and belong to the Fabaceae family. They are winter annual weeds; thus, they complete their life cycle from October to March. It has been commonly observed that these weeds come in various flushes, starting before the soil preparation for wheat sowing, and reach maturity before the wheat crop is mature and ready for harvest. In this way, they leave their plant residues in the field while the wheat is growing and affect the wheat crop through their allelopathic releases. During the growing season they release allelochemicals through leaf leachates and root exudation and hinder germination as well as plant growth and development. These weeds contain various phenolic compounds (allelochemicals) viz. *V. sativa* (4-hydroxy-3-methoxybenzoic acid, p-coumaric acid and ferulic acid), *T. polycerata* (m-coumaric acid, 4-hydroxy-3-methoxybenzoic acid and syringic acid), *L. aphaca* (gallic acid, m-coumaric acid, caffeic acid and syringic acid), *M. polymorpha* (vanilic acid, m-coumaric acid, 4-hydroxy-3-methoxybenzoic acid and p-coumaric acid) and *M. indica* (4-hydroxy-3-methoxybenzoic acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, m-coumaric acid, p-coumaric acid, syringic acid and vanillic acid) (Zohaib et al., 2014a). Studies have shown the allelopathic effect of *V. sativa* (Mukhtar and Bajwa, 2011; Zohaib et al., 2014b), *T. polycerata* (Zohaib et al., 2014a), *L. aphaca* (Om et al., 2002), *M. polymorpha* (Khan et al., 2012) and *M. indica* (Alam et al., 2002) on the germination and growth of different plant species; however, no literature is available that could reveal their allelopathic effects on wheat. Therefore, the present study was accomplished to ascertain the damaging effects of water extracts and decomposing residues of these weeds on wheat, which may provide an insight in to determination of levels of harm caused by these weeds to wheat productivity. Moreover, it may be helpful in devising weed control strategies.

MATERIALS AND METHODS

Experimental details

A twice repeated study was conducted to assess the allelopathic effect of some associated weeds of wheat *viz. V. sativa*, *T. polycerata*, *L. aphaca*, *M. polymorpha* and *M. indica* on germinability and biomass production of wheat seedlings in the laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.

Preparation of water extracts and residues of weeds

The plants of *V. sativa*, *T. polycerata*, *L. aphaca*, *M. polymorpha* and *M. indica* were collected by uprooting from wheat fields at maturity, throughout 2013. The collected weeds were dried at room temperature (21-23 ^{o}C) followed by oven drying at 70 ^{o}C for 24 hours. The small pieces of plants (1-3 cm) were made and soaked in distilled water at a 1:20 (w/v) ratio for 24 hours at room temperature. The extracts were filtered using a sterile metal tea strainer with a mesh size of 0.5 mm and then with Whattman No. 1 filter paper to remove impurity from the water extracts. The water extracts were stored in separate bottles and then tagged. These extracts were used as stock solutions of 5% (w/v) concentration and diluted to 2.5% (w/v) concentration for conducting the experiment at two different concentrations. On the other hand, the small pieces (1-3 cm) of weeds cut with the help of scissors were used as residues to check the effect of weed residues against wheat germinability and biomass production.

Experiment 1: Aallelopathic effect of water extracts of some weeds on germinability and biomass production of wheat seedlings

The experiment was carried out by using water extracts of *V. sativa*, *T. polycerata*, *L. aphaca*, *M. polymorpha* and *M. indica* at 2.5% (1:40 w/v) and 5% (1:20 w/v) concentrations to check their allelopathic effect on biomass production of wheat. Twenty-five wheat seeds were placed over double layer filter papers in each Petri dish (9 cm diameter). At the start of experiment, 7 mL of water extracts and distilled water was poured in each petri dish according to the nature of treatments. The distilled water was used as control for comparison. The experiment was laid out by using a completely randomized design (CRD) with factorial arrangement and four replications for each treatment. The average laboratory temperature during the whole period of experiment ranged from 21.8 to 23.2 °C. The experiment was observed for 16 d and Petri dishes were kept moist during the entire period of study using water extracts and distilled water according to the treatments.

Experiment 2: allelopathic effect of decomposing residues of some weeds on emergence and biomass production of wheat seedlings

Residues of *V. sativa*, *T. polycerata*, *L. aphaca*, *M. polymorpha* and *M. indica* at 2% (w/w) concentration were incorporated into the soil with different decomposition durations *i.e.* 0, 15 and 30 d. Soil-free weed residues were used as control. Twenty-five wheat seeds were sown in each pot. During the whole period of experiment, the soil was kept moist with distilled water. The experiment was laid out by using a completely randomized design (CRD) with factorial arrangement and four replications for each treatment. The average laboratory temperature during the course of the study ranged from 20.1 to 22.0 °C. The experiment was observed for 16 d.

Data collection

The number of germinated seeds/emerged seedlings was counted daily by using the procedure of seedling evaluation in the Handbook of Association of Official Seed Analysts (AOSA, 1990). A seed was counted as germinated when radical size reached up to 2 mm. Energy of germination/emergence (EE) was calculated using the formula of Farooq et al. (2006);

$$EE\ (\%) = \left(\frac{No.seeds\ \frac{germinated}{emerged}\ 4\ \text{d after sowing}}{Total\ No.of\ seeds\ tested}\right) \times 100$$

Time taken to 50% germination/emergence (T_{50}) was computed according to the formula of Coolbear et al. (1984) modified by Farooq et al. (2005):

$$T_{50}\ (\text{d}) = t_i + \left[\frac{N/_2 - n_i}{n_j - n_i}\right](t_j - t_i)$$

where, N is the number of germinated/emerged seeds, and n_i and n_j are the cumulative number of seeds germinated/emerged by adjacent counts at times t_i and t_j, respectively, when $n_i < N/2 < n_j$.

At the end of each experiment, wheat seedlings were uprooted and separated into shoots and roots after rinsing with tap water and drying with filter paper to determine biomass production. Fresh shoot and root biomass was determined by weighing immediately after uprooting with the help of an electric weighing balance. Then, the shoots and roots of wheat seedlings were dried in an oven at 70 °C to determine dry biomass. Root/shoot ratio of wheat seedlings was calculated by using following formula;

$$\frac{Root}{shoot} ratio = \frac{Root\ dry\ biomass}{Shoot\ dry\ biomass}$$

Statistical analysis

The data of both repetitions from each experiment showed a similar trend regarding all parameters, therefore data were pooled to calculate their average. The average data were analyzed using Fisher's analysis of variance (ANOVA) technique, and the least significant difference test (LSD) was used for the comparison of treatments' means at 0.05 probability level. Correlation analysis was performed to determine the association strength between different variables (Steel et al., 1997).

RESULTS AND DISCUSSION

Allelopathic effect of water extracts of weeds on germinability and biomass production of wheat seedlings

The aqueous extracts of weeds at varying concentrations exerted an inhibitory allelopathic influence on germination and biomass production of wheat seedlings as compared to the untreated control (Tables 1 and 2). It was found that energy of germination and time to complete 50% germination was significantly affected by the water extracts of all weeds. Maximum decrease in energy of germination (81%) and increase in time to 50% germination (226%) was caused by *M. indica* and *V. sativa* water extracts each at 5% concentration, respectively, as compared to the control. However, the water extracts of *V. sativa*, *L. aphaca* and *M. polymorpha* each at 5% concentration exhibited statistically similar results for energy of germination (Table 1). Water extracts of weeds significantly affected shoot and root fresh biomass of wheat seedlings. Shoot fresh biomass was increased by treatment with water extracts at 2.5% concentration while it decreased at 5% concentration as compared to control. The highest increase in shoot fresh biomass (30%) was observed in response to water extract of *M. indica* at 2.5% concentration while the lowest shoot fresh biomass (8%) was noticed under the influence of *T. polycerata* extract at 5% concentration. Root fresh biomass was reduced by water extracts of all weeds at both concentrations as compared to the control. The most inhibitory effect on root fresh biomass (64%) was caused by *M. indica* water extract at 5% concentration, as compared to control; by contrast, the effect of *L. aphaca* and *M. polymorpha* at 5% concentration was statistically similar (Table 1). Shoot as well as root dry biomass production was significantly affected by the water extracts of weeds. Shoot dry biomass was increased by the influence of the extracts of all weeds

Table 1 - Allelopathic influence of aqueous extracts of some weeds at different concentration levels on germination and fresh biomass production of wheat seedlings

Treatments	Germination energy (%)		Time to 50% germination (d)		Shoot fresh biomass (mg)		Root fresh biomass (mg)	
	2.5%	5%	2.5%	5%	2.5%	5%	2.5%	5%
Ck	90.00±4.08 a	90.00±4.08 a	0.98±0.05 g	0.98±0.05 g	101.41±0.93 de	101.41±0.93 de	53.23±0.30 a	53.23±0.30 a
W_1	67.50±2.50 b	25.00±2.89 d	2.03±0.20 de	3.19±0.12 a	127.20±2.37 ab	107.71±4.09 cd	42.32±1.91 b	27.49±0.63 f
W_2	82.50±4.79 a	47.50±4.79 c	1.63±0.05 f	2.54±0.17 b	111.09±1.87 c	93.58±3.34 f	37.98±0.69 c	26.85±1.16 f
W_3	60.00±4.08 b	25.00±2.89 d	1.87±0.05 def	2.19±0.19 cd	108.37±2.86 cd	96.46±2.56 ef	29.36±1.11 ef	21.13±0.66 g
W_4	62.50±2.50 b	22.50±4.79 d	1.71±0.05 ef	2.69±0.12 b	126.54±2.94 ab	120.46±2.3 b	34.35±1.32 d	21.94±0.85 g
W_5	65.00±6.45 b	17.50±2.50 d	2.36±0.08 bc	1.56±0.06 f	131.61±2.41 a	100.33±2.42 ef	30.40±0.49 e	19.42±0.72 g
LSD	11.580		0.327		7.361		2.735	

Values in the Table are means ± SE. Values with no similar letter differ significantly at the 0.05 probability level; Ck = control, W_1 = *Vicia sativa*, W_2 = *Trigonella polycerata*, W_3 = *Lathyrus aphaca*, W_4 = *Medicago polymorpha*, W_5 = *Melilotus indica*.

Table 2 - Allelopathic influence of aqueous extracts of some weeds at different concentration levels on dry biomass production of wheat seedlings

Treatments	Shoot dry biomass (mg)		Root dry biomass (mg)		Root/shoot ratio	
	2.5%	5%	2.5%	5%	2.5%	5%
Ck	10.99±0.43 e	10.99±0.43 e	6.06±0.19 a	6.06±0.19 a	0.55±0.02 a	0.55±0.02 a
W_1	12.85±0.35 bc	12.96±0.13 bc	5.29±0.22 b	3.98±0.13 cd	0.41±0.02 b	0.31±0.01 cde
W_2	13.19±0.14 b	12.57±0.31 bc	5.43±0.21 b	4.31±0.24 c	0.41±0.01 b	0.34±0.01 c
W_3	12.40±0.18 bc	12.15±0.29 cd	4.04±0.27 cd	4.10±0.24 cd	0.33±0.02 cd	0.34±0.01 c
W_4	13.12±0.17 b	11.43±0.34 de	3.81±0.22 cde	3.30±0.28 e	0.29±0.02 de	0.29±0.03 de
W_5	14.21±0.17 a	13.08±0.28 b	4.36±0.05 c	3.52±0.12 de	0.31±0.01 cde	0.27±0.01 e
LSD	0.828		0.595		0.047	

Values in the table are means ± SE. Values with no similar letter differ significantly at the 0.05 probability level; Ck = control, W_1 = *Vicia sativa*, W_2 = *Trigonella polycerata*, W_3 = *Lathyrus aphaca*, W_4 = *Medicago polymorpha*, W_5 = *Melilotus indica*.

at both concentrations, when compared with the control. Exaggeration in shoot dry biomass (29%) occurred when the treatment was applied with the water extract of *M. indica* at 2.5% concentration, as compared to the control. However, root dry biomass was reduced by the influence of water extracts of all weeds at both concentrations when compared with the control. Maximum decrease in root dry biomass (46%) occurred by the effect of *M. polymorpha* extract at 5% concentration; however, the effect of 5% *M. indica* water extract was statistically similar but with a slightly lower effect. Root/shoot ratio was decreased in response to the water extracts of weeds and the lowest root/shoot ratio (51%) was noticed by the influence of *M. indica* extract at 5% concentration, while the effect of *M. polymorpha* at both concentrations was statistically similar with a slightly lower effect (Table 2). Biomass production was positively associated with energy of germination while negatively correlated with time to 50% germination. However, it was observed that shoot biomass production did not correlate with germinability while both root biomass production and root/shoot ratio were correlated (Table 3).

Table 3 - Pearson's correlation coefficients indicating association strength between different variables under the influence of water. Extracts of weeds

	Germination energy	Time to 50% emergence	Shoot fresh biomass	Root fresh biomass	Shoot dry biomass	Root dry biomass	Root/shoot ratio
Germination energy	1	-	-	-	-	-	-
Time to 50% germination	-0.6751**	1	-	-	-	-	-
Shoot fresh biomass	0.1573^{NS}	0.1930^{NS}	1	-	-	-	-
Root fresh biomass	0.9046**	-0.6803*	0.0482^{NS}	1	-	-	-
Shoot dry biomass	-0.1825^{NS}	0.3971^{NS}	0.4777^{NS}	-0.4270^{NS}	1	-	-
Root dry biomass	0.8616**	-0.6455*	-0.1431^{NS}	0.9242**	-0.3769^{NS}	1	-
Root/shoot ratio	0.7818**	-0.6836*	-0.2727^{NS}	0.9161**	-0.6287*	0.9538**	1

^{NS,} *, ** indicate non-significance and significance at the 0.05 and 0.01 probability levels, respectively.

Allelopathic effect of weed residues on emergence and biomass production of wheat seedlings

Decomposing weed residues with various decomposition periods caused inhibition of emergence and biomass production in wheat seedlings. Weed residues reduced the energy of emergence and prolonged time to 50% emergence as compared to the control. The highest decrease in energy of emergence (85%) and delay in time to 50% emergence (138%) of wheat was ensued by *L. aphaca* and *M. polymorpha* residues decomposed for 30 d, respectively, as compared to the control. However, residues of *T. polycerata* and *M. polymorpha* decomposed for 30 d produced similar results regarding energy of emergence. Shoot and root fresh biomass production was perturbed by decomposing weed residues when compared with the control. The residues of *M. indica* decomposed for 30 d and *V. sativa* residues without decomposition caused maximum allelopathic inhibition of shoot fresh biomass (41%) and root fresh biomass (64%) production, respectively (Table 4). Shoot and root dry biomass production was substantially declined by the allelopathic effect of weed residues. When compared with control, maximum inhibition of shoot dry biomass (26%) production occurred by the influence of *M. polymorpha* residues decomposed for 30 d, while, the effect of *V. sativa* residues with a 30 d decomposition period was statistically similar with a slightly lower effect. On the other hand, root dry biomass (64%) production was hindered most by the allelopathic effect of *V. sativa* residues without any decomposition. Root/shoot ratio of wheat seedlings was decreased in response to weeds' residues toxicity as compared to control. However, in some instances, there was also an increase in root/shoot ratio by the influence of weed

Table 4 - Allelopathic influence of residues of some weeds with different decomposition periods on emergence and fresh biomass production of wheat seedlings

Treatments	Energy of emergence (%)			Time to 50% emergence (d)			Shoot fresh biomass (mg)			Root fresh biomass (mg)		
	0 d	15 d	30 d	0 d	15 d	30 d	0 d	15 d	30 d	0 d	15 d	30 d
Ck	67.50±4.79 a	67.50±4.79 a	67.50±4.79 a	3.18±0.24 i	3.18±0.24 i	3.18±0.24 i	173.57±1.80 a	173.57±1.80 a	173.57±1.80 a	110.00±2.27 a	110.00±2.27 a	110.00±2.27 a
W_1	17.50±2.50 def	17.50±6.29 def	15.00±2.89 ef	5.23±0.21 def	6.51±0.22 b	6.31±0.23 bc	138.22±1.33 cd	131.32±6.47 de	128.13±2.15 ef	39.58±1.73 j	78.96±2.41 def	65.92±3.39 h
W_2	27.50±4.79 cd	20.00±4.08 c-f	12.50±2.50 f	4.60±0.32 fg	4.58±0.10 fg	4.31±0.19 gh	136.19±2.12 cde	155.73±4.94 b	141.25±4.47 c	56.52±2.26 i	80.21±3.82 def	70.25±1.86 gh
W_3	30.00±4.08 c	52.50±4.79 b	10.00±0.01 f	4.59±0.19 fg	3.44±0.09 i	5.46±0.08 de	138.81±1.17 cd	159.68±2.80 b	157.46±1.39 b	84.13±1.76 cde	91.15±2.26 bc	87.31±5.24 cd
W_4	25.00±2.89 cde	15.00±2.89 ef	12.50±2.50 f	4.77±0.14 efg	5.73±0.32 cd	7.56±0.21 a	127.56±1.18 ef	157.48±3.70 b	120.31±2.19 f	55.98±1.38 i	55.99±2.60 i	73.46±3.46 fgh
W_5	20.00±4.08 c-f	42.50±6.29 b	15.00±2.89 ef	4.61±0.13 fg	3.75±0.32 hi	5.27±0.52 def	139.06±3.23 cd	175.04±3.25 a	102.28±3.24 g	78.92±3.25 ef	98.99±3.80 b	76.85±4.02 efg
LSD	11.495			0.693			8.699			8.360		

Values in the Table are means ± SE. Values with no similar letter differ significantly at the 0.05 probability level; Ck = control, W_1 = *Vicia sativa*, W_2 = *Trigonella polycerata*, W_3 = *Lathyrus aphaca*, W_4 = *Medicago polymorpha*, W_5 = *Melilotus indica*.

residues in plants growing in untreated control soil. The lowest root/shoot ratio was noticed by the effect of *V. sativa* residues without decomposition which was at par with *T. polycerata* residues without decomposition, while the highest root/shoot ratio occurred when wheat seedlings were treated with *T. polycerata* and *M. polymorpha* residues with a 30 d decomposition period (Table 5). The association between fresh as well as dry biomass was positive with energy of emergence and negative with time to 50% emergence. The relationship between root/shoot ratio and energy of emergence, time to 50% emergence and shoot dry biomass was non-significant while significantly different for root dry biomass, indicating that under the influence of allelopathic weed residues, root dry biomass production is more affected as compared to shoot dry biomass production (Table 6).

Allelopathic effect of water extracts of weeds on germinability and biomass production of wheat seedlings

The water extracts of all weeds inhibited germinability, shoot fresh biomass, and root fresh and dry biomass production except for dry biomass, which was enhanced when compared with the control; moreover, biomass production was positively correlated with energy of germination and negatively correlated with time to 50% germination (Tables 1-3). Inhibition of germinability and biomass production might be attributed to the water soluble phenolics present in the water extracts of weeds (Zohaib et al., 2014a). Reduced germinability might have occurred as a result of perturbed hormonal balance, protein synthesis, enzyme activity, photosynthesis and respiration (Zohaib et al., 2016). It has been observed that depressed and delayed germination by allelochemicals (phenolics) happens due to the suppression of respiration by hindrance of respiratory enzymes and the enzymes involved in oxidative pentose phosphate pathway (Muscolo et al., 2001) which is necessary for synthesis of RNA and DNA, production of ATP and generation of intermediates for Calvin cycle in early stages of greening of leaves during seedling germination (Taiz and Zeiger, 2002). Furthermore, inhibition of germination rate might also be due to alteration in gibberellic acid activity (Olofsdotter, 1998) which regulates the *de novo* synthesis of amylase during the germination process (Chandler et al., 1984). The decrease in biomass production occurs because of hindered cell division and photosynthetic activity owing to destruction of chlorophyll content (Shao-Lin et al., 2004). The inhibitory effect of various plant water soluble phenolics against various plants – e.g., chlorogenic acid, ferulic acid, comaric acid, 4-hydroxy-3-methoxybenzoic acid, gallic acid m-comaric acid, p-comaric acid, vanilic acid, cafeic acid - has been documented in the literature (Rodzynkiewicz et al., 2006; Baratelli et al., 2012; Muzaffar et al., 2012; Abbas et al., 2014; Zohaib et al., 2014a; Tanveer et al., 2015). Similarly, Jinhu et al. (2012) reported that depending upon concentration, the leaf leachates of *Eupatorium adenophorum* exerted inhibitory as well as promoting allelopathic effects on germination and dry matter accumulation of *Amaranthus retroûexus* and *Chenopodium glaucum* seedlings. Similarly, Mehmood et al. (2014) found m-coumaric acid, 4-hydroxy-3- methoxybenzoic acid, p-coumaric acid, ferulic acid, chlorogenic acid, vanilic acid and gallic acid in *Alternanthera philoxeroides* and *A. sessilis* and revealed their phytotoxicity against germination, seedling growth and biomass production in rice.

Table 5 - Allelopathic influence of residues of some weeds with different decomposition periods on dry biomass production of wheat seedlings

Treatments	Shoot dry biomass (mg)			Root dry biomass (mg)			Root/shoot ratio (mg)		
	0 d	15 d	30 d	0 d	15 d	30 d	0 d	15 d	30 d
Ck	13.03±0.22 a	13.03±0.22 a	13.03±0.22 a	11.32±0.34 a	11.32±0.34 a	11.32±0.34 a	0.87±0.04 b	0.87±0.04 b	0.87±0.04 b
W_1	13.01±0.33 ab	10.68±0.32 d-g	10.08±0.49 fg	4.06±0.28 g	5.86±0.33 f	7.81±0.28 de	0.31±0.03 i	0.55±0.03 fg	0.78±0.04 bc
W_2	13.12±0.27 a	12.28±0.54 abc	10.88±0.66 def	5.40±0.15 f	7.22±0.30 e	11.25±0.32 a	0.41±0.01 hi	0.59±0.04 def	1.05±0.07 a
W_3	10.96±0.79 def	11.76±0.43 bcd	12.28±0.24 abc	7.21±0.23 e	8.28±0.28 d	9.56±0.42 c	0.67±0.04 de	0.71±0.03 cd	0.78±0.02 bc
W_4	10.96±.039 def	10.88±0.62 def	9.60±0.53 g	5.20±0.24 f	5.84±0.17 f	10.00±0.44 bc	0.48±0.03 gh	0.54±0.03 fg	1.05±0.07 a
W_5	11.63±0.43 cde	12.92±0.39 ab	10.42±0.34 efg	5.94±0.33 f	10.62±0.51 ab	6.08±0.32 f	0.51±0.02 fgh	0.82±0.04 b	0.59±0.04 def
LSD	1.257			0.921			0.112		

Values in the table are means ± SE. Values with no similar letter differ significantly at 0.05 probability level; Ck = control, W_1 = *Vicia sativa*, W_2 = *Trigonella polycerata*, W_3 = *Lathyrus aphaca*, W_4 = *Medicago polymorpha*, W_5 = *Melilotus indica*.

Table 6 - Pearson's correlation coefficients indicating association strength between different variables under the influence of weed residues

	Energy of emergence	Time to 50% emergence	Shoot fresh biomass	Root fresh biomass	Shoot dry biomass	Root dry biomass	Root/shoot ratio
Energy of emergence	1	-	-	-	-	-	-
Time to 50% emergence	-0.8051**	1	-	-	-	-	-
Shoot fresh biomass	0.7168**	-0.7016**	1	-	-	-	-
Root fresh biomass	0.7582**	-0.5894*	0.6400**	1	-	-	-
Shoot dry biomass	0.6206**	-0.7358**	0.7271**	0.3621^{NS}	1	-	-
Root dry biomass	0.5523*	-0.4128^{NS}	0.5882*	0.7808**	0.2158^{NS}	1	
Root/shoot ratio	0.2925^{NS}	-0.1119^{NS}	0.2964^{NS}	0.6038**	-0.1536^{NS}	0.9256**	1

NS, *, ** indicate non-significance and significance at the 0.05 and 0.01 probability levels, respectively.

In present study, there was a differential allelopathic effect of different weeds on germinability and biomass production in wheat seedlings (Tables 1 and 2). This differential allelopathic effect might be due to the presence of different types of allelochemicals (phenolics) in water extracts of different weeds that acted differently (Zohaib et al., 2014a). Similar results were reported by Rahimzadeh et al. (2012), who noticed the inhibitory effect of water extracts of different weeds *viz.* red root amaranth, field bindweed and goosefoot weed against germination rate percentage and rate, and dry biomass of lentil and among different weeds goosefoot imposed the most inhibitory effect. In our study, the germinability and root biomass was inhibited by water extracts at both concentrations however shoot fresh biomass was inhibited at 5% concentration while enhanced at 2.5% concentration, when compared with control. On the other hand, shoot dry biomass production was enhanced at both concentrations, as compared to control. The correlation analysis also showed that the reduction in root/shoot ratio was the result of inhibition of root dry biomass production rather than shoot dry biomass (Tables 1-3). The inhibition of germinability at both concentrations shows that germination is much more sensitive to even lower concentrations of allelochemicals. Furthermore, the increase in shoot fresh and dry biomass at 2.5% concertation indicates the stimulatory effect of water extracts at a lower concentration (Salam et al., 2011). However, the increase in shoot biomass at a higher concentration and reduction in root biomass production at both concentrations might be due to the fact that root growth is exceedingly susceptible to the presence of allelochemicals in the rhizosphere (Baziramakenga et al., 1995) because roots tissues are much more permeable to allelochemicals as compared to shoot tissues (Nishida et al., 2005) which therefore may result in impaired root metabolic events and cell division in the root tips. Similar results were found by Ahmed et al. (2007): the water extract of *Lantana camara* caused inhibition of root growth of *Brassica juncea*, *Cucumis sativus, Phaseolus mungo*, *Raphanus sativus*, *Vigna unguiculata* and *Cicer arietinum* at all concentrations; however, shoot growth was enhanced in some instances at lower extract concentrations as compared to control.

Allelopathic effect of weed residues on emergence and biomass production of wheat seedlings

Residues of all weeds inhibited emergence as well as shoot and root biomass production in wheat seedlings (Tables 4 and 5). This inhibition might be due to the release of allelochemicals and their accumulation in soil from decomposing residues of weeds (Narwal et al., 2005). Similarly to our results, Ismail and Siddique (2012) reported that residues of *Fimbristylis miliacea* at 0.5, 1 and 2% concentration caused inhibition of germination and biomass production in rice plants as compared to control. Katoch et al. (2012) noticed that residues of *E. adenophorum*, *Ageratum conyzoides* and *L. camara* caused inhibition of germination and biomass production of wheat, rice and maize.

Differential allelopathic effects of decomposing residues of different weeds were noticed regarding emergence and root and shoot biomass production. Maximum inhibition of emergence was caused by residues of *M. polymorpha*, while shoot and root biomass production was inhibited by *M. indica* and *V. sativa* residues, respectively, as compared to control (Tables 4 and 5). This differential behavior might be due to the presence of different phenolic compounds in different weed tissues, differential release of phenolics from different weeds upon decomposition, and plant structure and decomposition pattern as well (Alam and Shereen, 2002; Bonanomi et al., 2011; Zohaib et al., 2014a). Similar results were found by Jalageri et al. (2010), who stated that residues of *Cyperus rotundus, Commelina benghalensis, Prosopis juliflora* and *Parthenium hysterophorus* exhibited inhibition of germination and biomass production of sorghum, soybean and ground nut. However, among various weeds, the inhibitory effect of *C. rotundus* and *C. benghalensis* was more pronounced.

In the present study, the allelopathic effect of residues of different weeds varied with decomposition period. Emergence and shoot biomass were inhibited mostly by residue decomposition for 30 d, while, root biomass was inhibited by residues without decomposition (Tables 4 and 5). Inhibition of emergence and shoot biomass by residues with 30 d decomposition might be due to the fact that with increasing time of residue decomposition, there occurs accumulation of allelochemicals in soil (Narwal et al., 2005); furthermore, in some instances, decomposition products of allelochemicals become more toxic than the original allelochemicals themselves (Albuquerque et al., 2011). Chou et al. (1976) reported the release of 18 allelochemicals from decomposing corn residues and 9 allelochemicals from rye residues in the soil after decomposition for 30 d. Some of those allelochemicals were found to be moderately toxic to lettuce growth while others were noticed to be highly toxic. Similarly, An et al. (2001) found an increase in accumulation of allelochemicals in soil upon increase in the decomposition period of *Vulpia myuros* residues. However, in our study, a more toxic effect of residues on emergence and shoot biomass production of wheat occurred when residues were decomposed for 30 d while root biomass was inhibited mostly by residues without decomposition and the inhibition increased up to 30 d (Tables 4 and 5). This indicates that under the influence of allelopathy, more biomass is partitioned to the roots for proper root growth to occur and support the plant. This was further evidenced by the fact that root/shoot ratio and correlation analysis showed a positive relationship between root dry biomass and root shoot ratio (Table 6). This decreased inhibition of root biomass production might be due to reduced respiration losses in roots because of hindrance in activities of respiratory enzymes caused by allelochemicals (Zohaib et al., 2016).

The results revealed that the water extracts of weeds at different concentrations and residues with different decomposition periods inhibited the germinability and biomass production of wheat seedlings. A higher water extract concentration posed a more inhibitory effect than a lower concentration on germinability and biomass production. Weed residues with 30 d of decomposition caused more inhibition of emergence and shoot biomass production and residues without decomposition caused more inhibition of root biomass, indicating that decomposing weed residues are equally dangerous after each decomposition period to the events of emergence and growth of plants. The results conclude that leachates and decomposing residues of weeds caused inhibition of wheat germinability and biomass production; therefore, these weeds should be eradicated from wheat fields at early stages.

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