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ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACTS OF SUNFLOWER ON WHEAT (*TRITICUM AESTIVUM* L.) AND MAIZE (*ZEA MAYS* L.)

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Abstract

Sunflower is a potent allelopathic plant which possesses important allelochemicals with known allelopathic activity on other plants. In this study, allelopathic effects of fresh aqueous extracts (FAE) and air dried aqueous extracts (DAE) of root, shoot and leaves of sunflower (*Halianthus annuus* L.) were investigated on germination and seedling growth of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) in seed bioassay experiments carried out at Botany Department of Peshawar University during 2010. Results showed significantly inhibitory effects of aqueous extracts on seed germination, growth and dry biomass of seedlings of wheat and maize. In wheat seedlings, significant germination inhibition (15.21%), increased mean germination time (MGT) (57.76%), reduced plumule and radical growth (21.66 and 28.44%) and lowered seedlings dry biomass (31.05%) were recorded under dry aqueous extracts of leaf when compared to control. Germination percentage of maize was inhibited by dry aqueous extracts of leaf by 7.81%, germination index by 16.51%, increased MGT by 25.53%, decreased plumule and radical lengths by 29.00 and 36.12% respectively, and lowered maize seedling dry biomass by 34.02 %. In both experiments, dry aqueous extracts (DAE) were more phytotoxic than fresh aqueous extracts (FAE). Similarly, inhibitory effects of aqueous extracts of different parts of sunflower were recorded in the order leaf > shoot > root for both tested plants.

Key words: Allelopathy, Competition, Crop rotation, Plant interactions, Secondary metabolites.

Introduction

Sunflower (*Halianthus annuus* L.) of family Asteraceae is a potential allelopathic plant possessing a number of bioactive allelochemicals which show allelopathic effects on other plants (Macias *et al*., 2002). Principal allelochemicals of sunflower are phenolic compounds, flavonoids and terpenoids (Macias *et al*., 2004; Gawronska *et al*., 2007). These compounds when leached to the surrounding rhyzosphere, strongly interfere with germination and growth of other plants and may correspond to modification of neighboring flora in several ways. In general, allelochemicals are released to the surrounding environment through rain leachates, exudates or as residual components (Ashrafi *et al*., 2008), which then influence plants by inhibitory or stimulatory allelopathic activity, generally depending on the nature and concentration of released allelochemicals (Bhowmik & Inderjiit, 2003; Muhammad *et al*., 2013).

Allelopathic effects of sunflower on other crops and weeds are well established in the literature (Mahmood *et al*., 2013). Low germination rate of maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* Mill), soybean (*Glycine max* L.) and bean (*Phaseolus vulgaris* L) was observed in response to aqueous extracts of aerial parts and root of sunflower (Beltran *et al*., 1997). Aqueous extracts of sunflower in combination with aqueous extracts of sorghum resulted in reduction of biomass of different weeds (*Rumex dentatis* L., *Chenopodium album* L., *Coronopus didymus* (L.) Sm., *Fumaria parviflora* Lam.) and corresponded to yield increase of wheat (Cheema *et al*., 1997). Batish *et al*., (2002) documented reduced growth and yield of *Cyamopsis tetragonoloba, Sorghum vulgare*, *Pennisetum americanum* and *Zea mays* grown in fields containing residues of sunflower. Leaf extracts of sunflower at different concentrations when applied to mustard (*Sinapis alba* L.) showed inhibitory effects on seed germination and seedling growth of tested plant (Bogatek *et al*., 2006). Water extracts and residues of sunflower significantly reduced seed germination, seedling growth and biomass of wild barely (Ashrafi *et al*., 2008). Different concentrations of aqueous extracts of sunflower significantly decreased biomass of four wheat varieties showing strong allelopathic activity of sunflower (Anjum & Bajwa, 2010). Nikneshan *et al.,* (2011) studied different concentrations of air-dried leaf powder of eight cultivars of sunflower for allelopathic activities on wheat and other target plants. They reported decrease in germination indices of wheat and other tested plants at higher concentrations. Different aqueous extracts of sunflower and other plants reduced root and shoot length, biomass accumulation, chlorophyll contents and lateral plant spread when applied to weeds of rice, grasses and sedges (Khaliq *et al.*, 2013).

In Pakistan, sunflower is grown in rotation with different crops predominantly with wheat, maize, rice, sugarcane, soybean and cotton in different sequence. Rotation sequence of sunflower with different crops is different and is dependent on sunflower and subsequent crop cultivars i.e., spring or autumn varieties. Therefore it is important to understand allelopathic activities of sunflower on subsequent crops for modification of rotational sequence and for better yields. The aim of this study was to investigate the allelopathic effects of fresh and air-dried aqueous extracts of different plant parts (root, stem and leaves) of sunflower on wheat and maize in seed bioassay experiment.

Materials and Methods

Extracts preparation: Leaves, shoots and roots of fully mature sunflower (cv. Hyssun-38) were collected from experimental fields of Agriculture University, Peshawar in July 2010. In order to remove dust and soil, plant parts were thoroughly washed with tap water and then dried with tissue paper. For preparation of fresh aqueous extracts, 10 gram of each sample (root, shoot and leaves) were crushed in a blender and soaked in 100 ml distilled water for 12 hours (Hussain *et al*., 2010). Crushed materials were filtered through four layer muslin cloth and then re-filtered through Wattman No. 1 filter paper. The filtrate was considered as original stock solution

(10%) and stored in a refrigerator at 4ºC for further use. Dried aqueous extracts were prepared by drying root, shoot and leaves in air at shade for seven days. 10 % dry aqueous extract was obtained from each part i.e., root, shoot ant leaves following the same method as described for fresh aqueous extracts.

Seed bioassay: Seed bioassay was performed during July 2010 at the Department of Botany, University of Peshawar under room conditions at temperature range 20-25°C. Seeds of wheat (*Triticum aestivum* L.) cv Fakhar-e-Sarhad and maize (*Zea mays* L.) cv Sadaf were obtained from the Herbarium of Botany Department, University of Peshawar. Ten seeds each of maize and wheat were grown in petridishes containing double folded Wattman No. 1 filter paper. Each petridish was provided with 10 ml of respective extracts i.e., fresh aqueous extract (FAR) and dry aqueous extract (DAE) of root, shoot and leaf. 10 ml of distilled water was used as control. Each treatment was replicated four times and the experiment was arranged in completely randomized design (CRD). Data for germination percentage, mean germination time, germination index, radical and plumule length and seedling dry biomass was recorded for ten days after the establishment of experiment. Germination percentage was determined by counting germinated seeds after 24 hours of sowing till 10th day. Mean germination time (MGT) was calculated by relation $\Sigma (n_i \times t_i)/\Sigma n_i$ (Hu *et al.*, 2005). Where n_i corresponds to number of seeds germinated on ith day while t_i is time taken in days. Germination index (GI) was measured according to method described by Association of Official Seed Analysts (Anon., 1999).

Data on the studied parameters was subjected to analysis of variance (ANOVA) and differences between means for significance were calculated by least significant difference (LSD) at $p \leq 0.05$. Computer software SPSS was used for statistical analysis (Anon., 2012).

Results and Discussion

Results of this study demonstrated significant effects of both fresh and dry aqueous extracts of sunflower's leaves, shoot and root on the germination, seedling growth and seedling dry biomass of wheat and maize. Germination parameters, radical and plumule growth and seedling dry biomass of wheat was strongly influenced by both fresh and dried aqueous extracts; however, dry aqueous extract was more phytotoxic than fresh aqueous extract of the corresponding plant parts. Among the tested plant parts of sunflower for allelopathy, both fresh and dried aqueous extracts of leaves showed highest degree of phytotoxicity affecting all stages of germination and growth of wheat seedling. Results showed that dry aqueous extracts of leaves reduced germination by 15.21%, GI by 45.53%, plumule length by 14.62%, radical length by 28.08% and seedling dry biomass by 15.52% of the control; MGT was significantly increased (57.76%) as shown in Table 1. Similar but comparatively less phytotoxic effects were recorded for fresh and dry aqueous extracts of shoot which caused significant reduction in germination percentage, GI, radical and plumule lengths and seedling dry biomass; however, increase in MGT of wheat. Fresh aqueous extracts (FAE) of roots did not cause any significant effect on wheat seedling, however, dry aqueous extracts (DAE) resulted in slightly reduced germination percentage (2.09%), increased MGT (11.37%) and reduced germination index (8.92%) when compared to control (Table 1).

Table 1. Effects of fresh and dry aqueous extracts of root, shoot and leaf of sunflower on germination and growth of wheat. Seedling dry biomass is average of ten plants.

Plant parts	Germination (%)	MGT (days)	Germination index (GI)	Plumule length (cm)	Radical length (cm)	Seedling dry biomass (g)
	Fresh aqueous extracts (FAE)					
Control	99.25	4.57	5.49	16.34	13.92	0.219
Root	99.03	4.58	5.47	16.30	13.88	0.218
	(-0.22)	(+0.21)	(-0.36)	(-0.24)	(-0.28)	(-0.45)
Shoot	93.07*	5.97*	4.29*	14.87*	11.49*	0.196*
	(-6.22)	(+30.63)	(-21.65)	(-8.99)	(-17.45)	(-10.50)
Leaf	87.10*	7.21*	2.99*	13.95*	10.01*	0.185*
	(-12.24)	(+57.76)	(-45.53)	(-14.62)	(-28.08)	(-15.52)
LSD value	**14.12**	**1.31**	**0.62**	**1.21**	**1.80**	**0.013**
	Dry aqueous extracts (DAE)					
Control	99.25	4.57	5.49	16.34	13.92	0.219
Root	97.17*	5.09*	5.00*	16.27	13.80	0.215
	(-2.09)	(+11.37)	(-8.92)	(-0.42)	(-0.86)	(-1.82)
Shoot	92.01*	6.01*	4.03*	13.09*	11.00*	0.178*
	(-7.29)	(+31.50)	(-26.59)	(-19.88)	(-20.97)	(-18.72)
Leaf	84.15*	7.13*	2.17*	12.80*	9.96*	0.151*
	(-15.21)	(+56.01)	(-60.47)	(-21.66)	(-28.44)	(-31.05)
LSD value	**1.871**	**0.404**	**0.321**	**2.852**	**1.120**	**0.027**

*Significantly different from control at $p \leq 0.05$; values in parenthesis represent percent increase (+) or decrease (-) of control

Table 2. Effects of fresh and dry aqueous extracts of root, shoot and leaf of sunflower on germination and growth of maize. Seedling dry biomass is average of ten plants.

Plant parts	Germination (%)	MGT (days)	Germination index (GI)	Plumule length (cm)	Radical length (cm)	Total dry biomass (g)
			Fresh aqueous extracts (FAE)			
Control	100	7.52	17.14	11.41	8.00	0.435
Root	100	7.52	17.13	11.37	7.95	0.430
	-	-	-	(-0.35)	(-0.62)	(-1.14)
Shoot	94.71*	8.42*	15.50*	10.03*	6.72*	0.380*
	(-5.29)	(+11.96)	(-9.50)	(-12.09)	(-16.00)	(-12.64)
Leaf	93.12*	9.01*	15.00*	8.79*	5.76*	0.301*
	(-6.87)	(+19.81)	(-12.48)	(-21.38)	(-28.00)	(-30.80)
LSD value	**1.540**	**0.641**	**1.234**	**0.981**	**1.100**	**0.022**
			Dry aqueous extracts (DAE)			
Control	100	7.52	17.14	11.41	8.00	0.435
Root	100	7.57	17.12	10.14*	7.20*	0.401*
	-	(+0.66)	(-0.11)	(-11.13)	(-9.99)	(-7.81)
Shoot	93.17*	8.64*	15.01*	9.80*	6.07*	0.311*
	(-6.82)	(+14.89)	(-12.42)	(-14.11)	(-24.37)	(-28.50)
Leaf	92.19*	9.44*	14.31*	8.10*	5.11*	0.287*
	(-7.81)	(+25.53)	(-16.51)	(-29.00)	(-36.12)	(-34.02)
LSD value	**4.076**	**1.029**	**0.768**	**1.075**	**0.234**	**0.017**

*Significantly different from control at $p \leq 0.05$; values in parenthesis represent percent increase (+) or decrease (-) of control

Data on germination parameters and seedling growth of maize as affected by fresh and dry aqueous extracts of root, shoot and leaf of sunflower is presented in Table 2. Results showed that fresh aqueous extract (FAE) had no significant effects on maize seedling. However, DAE of root inhibited plumule, radical lengths and seedling dry biomass to significant extents. Compared to control, 94.71% seed germination, 8.42 MGT (days), 15.50 GI, 10.03 cm plumule length, 6.72 cm radical length and 0.380 g dry biomass were recorded in petridishes treated with FAE of shoot. Effects of dry aqueous extract of shoot on germination and growth of maize seedling were comparatively more inhibitory than fresh aqueous extracts. Both FAE and DAE of leaf once again showed strong inhibitory effects on germination (93.12% FAE; 92.19% DAE), MGT (9.01 days FAE; 9.44 days DAE), GI (15.00 FAE; 14.31 DAE), plumule length (8.79 cm FAE; 8.10 cm DAE), radical length (5.76 cm FAE; 5.11 cm DAE) and seedling dry weight (0.301 g FAE; 0.287 g DAE) respectively. Mean germination time (MGT) was significantly increased by FAE and DAE of leaf corresponding to 9.01 days and 9.44 days respectively (Table 2).

Sunflower is an allelopathic plant which possesses many phytotoxins with known allelopathic activity against weeds and many crops. The present study revealed strong allelopathic effects of sunflower on maize and wheat germination and seedling growth parameters. Generally, leave extracts were relatively more phytotoxic than other parts of sunflower used in this study. Also, differential toxicity was shown by fresh extracts and dry extracts. Phytotoxicity of sunflower parts was recorded in the order leaves > shoot > root and allelopathic influence of the nature of extracts was recorded as dry aqueous extracts > fresh aqueous extracts. Previously allelopathic activity of sunflower against wheat (Ghaffar *et al*., 2000; Bashir *et al.*, 2012), maize (Beltran *et al*., 1997) and weeds (Saeed *et al.,* 2011) in laboratory bioassay as well as in field experiments has been reported. Sunflower possess a number of allelochemicals predominantly alkaloids, phenolic compounds, flavonoids and terpenoids with confirmed allelopathic action against many plants (Macias *et al*., 2004; Gawronska *et al*., 2007). These chemicals are either secondary metabolites which are involved in modifying neighboring flora and microbes by either suppressive or stimulative allelopathy besides their active role in host defense against pathogens, herbivores, insects and pests. Allelochemicals often results in inhibition and delay in germination, reduced radical and plumule growth and low dry matter of competing plants (Herro & Callaway, 2003; Sajjad *et al*., 2007) possibly by altering cell division patterns, physiology, water and minerals uptake capacity of the receptor plants (Majeed *et al*., 2012). Thus reduced germination percentage, germination indices, delay in mean germination time, reduced growth of radical and plumule and low dry matter of wheat and maize seedling in this study may be attributed to suppressive effects of allelochemicals present in sunflower which could have possibly modified cell division, physiological functions and absorption capacity of target plant seedlings. It may be inferred that potential allelochemeicals in sunflower could have changed the cell membrane permeability of wheat and maize seeds in this study with reduced compilation of energy reserves during germination and distortion of receptors necessary for seed germination with subsequent decrease in germination and seedling growth (Rizvi & Rizvi, 1992) or it may be due to changes in respiration with reduced production of ATP, RNA synthesis or disturbance in functions of secondary messengers necessary for germination, seedling growth and biomass (Gatti *et al*., 2010). These results are in agreement with published reports on sunflower allelopathy against wheat and maize (Beltran *et al*., 1997; Anjum and Bajwa, 2010; Nikneshan *et al*., 2011) and are in general conformity with researchers elsewhere who reported allelopathic potentials of sunflower on weeds and other crops (Cheema *et al*., 1997; Batish *et al*., 2002; Ashrafi *et al*., 2008; Khaliq *et al*., 2013). There are many plant species that are potential candidates for allelopathic effects (Marwat *et al.*, 2008). Differential allelopathic activity of different parts of sunflower (root, shoot and leaf) on wheat and maize seedling in the present study may be related to the nature and concentration of allelochemicals present in different organs of plants. Plants leaves, generally, have more allelochemicals than other parts; thus more phytotoxic.

In conclusion, results of this study demonstrated allelopathic actions of water extracts of root, shoot and leaf against wheat and maize in the order leaf > shoot > root and DAE > FAE; corresponded to lower and delayed germination, germination indices, radical and plumule lengths and significantly lowered dry biomass of tested plant seedling in seed bioassay experiment. Field trials are suggested for further elucidation of allelopathic activity of sunflower on wheat and maize.

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