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ALLELOPATHIC POTENTIAL OF *CHENOPODIUM ALBUM* L.: EFFECTS OF AQUEOUS EXTRACT ON GERMINATION AND RADICLE GROWTH OF WHEAT AND MAIZE

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ABSTRACT

A laboratory trial was conducted to investigate the effect of lambsquarter *Chenopodium album* L. aqueous extract on germination and radicle elongation of two test species, namely wheat and maize. The trial was replicated five times with three concentrations of the weed extract including 2, 4, 6% and control. The germination and radicle length were recorded for ten days. Aqueous extract of *Chenopodium album* was strongly inhibitory to germination and had remarkable effect on the growth of wheat and maize. Final germination and speed of germination were both reduced by the extracts. Germination and root length of wheat and maize was significantly suppressed at higher concentration (i.e. 4 and 6%), while lower concentration (2%) of the extract in both test species showed greater germination and radicle elongation than at higher concentration. Thin layer chromatography of the phenolics present in the shoot was performed. Seven phenolic compounds were detected .which presumably act as the allelochemicals under field conditions. The possible mechanisms of germination and radicle growth inhibition are discussed.

Key-words: Allelopathy, *Chenopodium album*, wheat, maize.

INTRODUCTION

Allelopathy is the ability of plants to inhibit or stimulate growth of the neighboring plants in the environment by exuding chemicals. Although the term "Allelopathy" has undergone several changes, it has been redefined as "any direct or indirect harmful or beneficial effects by one plant on another through the production of chemical compounds that it releases into the environment (Shaukat *et al*., 1983, Birkett *et al.,* 2001). Allelopathy is a natural phenomenon that may be considered as a tool for biological weed control in crop production (Marcias *et al*., 2007; Heidarzadeh *et al*., 2010). Many secondary metabolites may function as allelochemicals, such as turpenoids, phenolics, alkaloids, fatty acids, steroids and polyacetylenes (Kohli *et al*., 2001; Blokker *et al*., 2006). The allelochemicals are released in soil by weeds and when the allelochemicals are accumulated in soil in significant amounts, they inhibit the processes of germination and growth and thereby reduce crop production. Shaukat *et al*., (2002) reported that whereas a 50% aqueous extract of *Argemone mexicana* promoted plant growth a 100% extract concentration significantly reduced plant height, and fresh weight of shoots and root. In general, decomposing plant material caused greater phytotoxicity compared with the aqueous extract. Similarly, Akhter *et al.,* (2001) reported that aqueous extracts of *Cirsium arvense* and *Ageratum conyzoides* could suppress the germination and early seedling growth of some weeds of wheat. Dahiya and Narwal (2003) found that root exudates of *Helianthus annus* L. are allelopathic towards *Agropyron repens* L., *Avena fatua* L., *Celosia crustata* and *Chenopodium album* L. Shaukat *et al.,* (1999) reported the effect of five phenolic compounds (allelochemicals) alone and in combination with 2, 4-D herbicide in sandy and clay loam soil and demonstrated the suppressive and synergistic effects of the allelochemicals and phenolic compounds at different depths. Some crop plants have weed suppressive activity such as *Sorghum bicolor* (L.) Moench. which is used as a natural herbicide. The allelopathic interference by weed extract has been established as one of the several factors that regulate the growth of plants (Alam *et al*., 1997).

Weeds are the unwanted plants having relatively little economic value and also grow in association with domesticated crops. They affect the crops in different steps of growth and compete for water, nutrients, light and other resources. Their seeds remain dormant for long periods and remain viable for many years. Weeds can be controlled manually, chemically and sometimes mechanically. The manual method is time consuming and laborious while the chemical control method is more effective than others. Sometime plants show the positive allelopathic effects on the cultivated crops like Sorgaab water extract alone and in combination with herbicides for controlling weeds in mung bean crop (Khaliq *et al*., 2002).

Lambsquarter (*Chenopodium album* L.)

Chenopodium album L. commonly known as Lambsquarter (family: Chenopodiaceae) is a cosmopolitan weed, commonly present throughout Pakistan mainly in tropics but also in cooler areas. It grows in gardens, ruderal places,

road sides, irrigated places and sub-humid places and grows in wide range of soil types and pH values. *Chenopodium album* as delimited here, is not uniform, its morphological variation is wide probably both because of genetic and environmental factors.

It is a most problematic weed of wheat which is proven by many surveys of agricultural fields in Pakistan. Le Tourneau *et al*., (1956) and Alam *et al.,* (2002) reported reduction in both shoot and root growth of wheat, when grown in 2% (W/V) extract of common lambsquarter. Bukolova, (1971) and Alam et al., (2002) found reduced mitotic activity in the roots of wheat, rye and garden cress following treatment with *Chenopodium* extract. Anjum and Bajwa (2010) reported the broad leaf weeds *Rumex dentatus* L. and *Chenopodium album* L. as the main cause of low yield of wheat in Pakistan. The effect on tillering capacity decreases by 41.6% and consequently, the crop yield. Alam *et al*., (2002) studied the effect of *Chenopodium album* extract alone or in combination with NaCl on growth of wheat crop. The weed and salinity together may be hazardous to the growth of crop. Germination was not significantly affected but root growth was more affected than shoot growth.

MATERIALS AND METHODS

Collection of samples

The allelopathic effects of aqueous extract of *Chenopodium album* on seed germination and radicle elongation of test species wheat and maize were investigated.. The field grown fresh plants of common lambsquarter (*Chenopodium album* L.) were collected from Federal Urdu University Campus, Gulshan-e-Iqbal, washed with distilled water and air dried in greenhouse for ten days. Trial was conducted in laboratory, Department of Botany, Federal Urdu University, Karachi. The well dried plant samples were ground in a Willey mill and passed through a 20 mesh screen. The seeds of wheat and maize were obtained from Pakistan Agricultural Research Council (PARC).

Germination and growth

The aqueous extract was prepared from ground material in different percentages i.e. 2%, 4% and 6%. Two, 4 and 6g of plant materiel were soaked in 100 ml distilled water for 24 h at room temperature. Subsequently, the aqueous extract was obtained by filtering the mixture using Whatman No. 1 filter paper. Distilled water served as control. Petri plates were thoroughly washed with detergents. Seeds of test species cleaned and surface sterilized with Sodium hypochlorite (2%). Ten seeds of maize or ten seeds of wheat were grown in Petri dishes. Five 5ml of 2%, 4% and 6% aqueous extract was poured into the dishes. Five ml distilled water served as control. Treatments and controls were replicated five times each. Small amounts of respective solutions were added when it was obvious that Petri plates were beginning to dry out. Germination was recorded daily and root (radical) growth was measured at 10 days.

Statistical analysis

Data were subjected to statistical analysis. Germination data of both the test species were analyzed by RMD (Repeated Measures Design) using SPSS Ver. 14 Software. The percentage of the total number of seeds of both test species which germinated during 10 days period was recorded. A "Speed of germination" index (S) for each species was determined by following formula (Khandakar and Bradbeer 1983).

$$S= \{{}^{N}/_{1}+{}^{N}/_{2}+{}^{N}/_{3}+\ldots..+{}^{N}/_{N}\}\times 100$$

Where N1, N2, N3….N=total number of seeds in a treatment which germinated on day 1,2,3…..N following setup of the experiment; the index S ranges from 0 (If no seed ever germinated) to 100 (If all seeds germinated on the first day). The program GERMSPD was developed by one of us (S.S.S.).

Chromatography:

Ether extract of *Chenopodium album* was evaporated to dryness, dissolved in 2 ml of 80% ethanol and used for loading on silica gel F_{254} thin layer chromatographic plate. The chromatogram was developed in acetic acid-chloroform 1: 9 v/v by ascending chromatography using reference phenolic compounds. The phenolic compounds were detected using ferric chloride –ferric cyanide [$FeCl_3.K_3Fe(CN)_6$] reagent and under UV-light along with ammonia vapors (Harborne, 1973). R_f values of the compounds were determined and matched with those provided in literature (e.g., Harborne, 1973).

RESULT AND DISCUSSION

Effect of aqueous extract of Chenopodium album on germination of wheat and maize:

Germination of the two test species was inhibited by various concentrations of the extract compared to controls and the degree of inhibition varied. In wheat seeds mean of percent germination reduce in higher concentration as compared with control (Table 1). Speed of germination was also slow in 6% extract of *Chenopodium album* (33). Similarly in maize the germination was highly affected by 6% extract mean of percent germination was (40). Normally the speed of germination in not fast in control (73%) but slow in treatment like in 6% was (35%) only. Similar observation was found by Ankita and Chabbi (2012) aqueous extract of weeds in different concentration were applied to determine their inhibitory effect on germination, seedling length and seedling dry and fresh weight of crops. Likewise, weed extract also affected the germination of maize seeds and the the degree of adverse effect was similar for the two test species. The maximum percentage was found in control (82%) and the minimum (42%) in 4% extract. Inhibition of germination of several crops has also been demonstrated when their seeds were exposed to root and shoot extracts of a weed, *Citrullus colocynthis* (Shaukat *et al.,* 1985).

Table 1. Effect of *Chenopodium album* extract on final germination percentage, speed of germination and radicle growth and reduction of radicle growth of wheat (*Triticum aestivum* L.).

Treatments	Final Germination (%)	Speed of Germination (%)	Radicle growth (???)	% reduction in radical growth
Control	94±2.44	86.6	12.02±0.71	-
2%	44±15.03	86	1.90±0.54	84.2
4%	56±10.77	49	1.16±0.13	90.35
6%	48±15.93	33	0.79±0.23	93.43

Table 2. Effect of *Chenopodium album* extract on final germination percentage, speed of germination and radicle growth and reduction of radicle growth of maize (*Zea mays* L.).

Treatments	Final Germination (%)	Speed of Germination (%)	Radicle growth (???)	% reduction in radical growth
Control	84±4	73	15.23±0.29	-
2%	68±8	61	4.74±0.5	68.8
4%	60±8.3	48	1.98±0.34	87
6%	40±8.3	35	1.66±0.23	89.2

Table 3. Results of Repeated Measures Design using Generalized Linear Model (GLIM) for the effect of *Chenopodium album* extract on germination of wheat and maize.

	Effect	Value	F	Hypothesis df	Error df	P
Wheat	Factor1 Pillai's Trace	0.373	14.293[a]	4	96	<0.001
	Wilk's Lambda	0.627	14.293[a]	4	96	<0.001
Maize	Factor1 Pillai's Trace	0.540	28.223[a]	4	96	<0.001
	Wilk's Lambda	0.460	28.223[a]	4	96	<0.001

Effect of weed extract on radicle elongation of wheat and maize:

The radicle elongation of wheat and maize (Table1and 2) was inhibited by the weed extract. The extent of reduction was at a greater degree for wheat compared to maize. Lovett *et al.*, (1989) also reported that biological activities of receiver plants to allelochemicals that the response is known to be concentration dependent. Responses are characteristically, stimulations at low concentration of allelochemicals and inhibition at the higher concentration. In wheat seedling was highly affected compared with control (Table1). Percent inhibition of radicle growth over

control highly increased in 6% (93.43%) and also in 2% and 4% extract. In maize radicle growth decreased in high concentration of extract (Table 2). Percent inhibition of radicle growth was the maximum in 6% of extract as compared with control. Suseelamma *et al*., (1992) found a significant reduction in root and shoot length of horse gram with the extract of *Digera muricata* and Shaukat *et al.* (1985) reported more inhibition of root growth of *Lactuca sativa, Sorghum bicolor, Brassica rapa, Pennisetum americanum* and *Medicago sativum* under influence of extracts of *Citrullus colocynthis*, a weed of several crops in Sindh.

Table 4. R_f values of phenolic principles in ether fraction of aqueous extract of *Chenopodium album* and reaction to chromatogram developing reagents.

Phenolics	Rf-value	$FeCl_3+K_3Fe(CN)_6$	UV + NH_3 vapours
Caffeic acid	68.71	Dark blue	Bright blue green
Vanillic acid	84.14	Purple	Blue green
Syringic acid	79.87	Purple	Blue
p-Coumaric acid	71.56	Blue	Bright violet
Ferulic acid	73.48	Purple blue	Blue green
Gallic acid	21.05	Brownish	Bluish-brown
Unknown	96.34	Bluish	Blue

Table 3 showed the different aqueous extracts significantly suppressed radicle elongation of both the test species. Because of the non-independence of observations, repeated measures design (RMD) was performed. Factorial analysis of variance (FANOVA) was not applicable and RMD was mandatory for this analysis. The result of repeated measures design ANOVA showed a significant effect of allelopathic treatment in wheat seeds ($F=61.25$, $P<0.001$). In the result of other test species maize the repeated measure design ANOVA showed a significant effect of extract ($F=98.5$, $P<0.001$). Furthermore the significance of the results of repeated measures can also be judged by significant values of Pillai's trace and Wilk's Lambda ($P<0.001$) in both the test species *i.e*, wheat and maize.

These results showed that the growth parameters of test species were significantly inhibited when water extract of the weed was applied to the test species. The toxic effects of the phenolic compounds on seed germination and plant growth have been previously reported (Stowe *et al*., 1987; Blum, 1996; Inderjit, 1998; Burhan and Shaukat, 2000). It is concluded that water extract of weed used in this study inhibit the germination and radical elongation of wheat and maize. Control and management of the weed is needed in crop field because the weeds not only interfere with crop growth but also adversely affect the biodiversity of the area mostly due to their allelopathic influence.

Allelochemicals:

Chromatograms sprayed with ferric-chloride-ferricyanide reagent and UV-rays disclosed seven different phenolic compounds from the shoot tissue of *Chenopodium album* (Table 4). These were: caffeic acid, vanillic acid, syringic acid, p-coumaric acid, ferulic acid, gallic acid and an unknown. Due to the death and decay of plants and as a result of leaching it is possible that these phenolic compounds may accumulate in biologically significant amounts in the soil and play a key role as habitat variable exerting a causative influence on growth and development of other neighbouring plants.. Although the roots of *C. album* were not tested for the phytotoxins, they also presumably release the diffusates into the soil. The toxic nature of phenolics to germination and growth of plants has been reported by many workers (Blum, 1996; Reigosa *et al*., 1999; Burhan and Shaukat, 2000; Stupnicka-Rodzynkiewicz *et al.*, 2006). Thus the inhibition of radicle growth by aqueous sextract of *Chenopodium album* can be expected to be due to phenolic compounds. Therefore, the allelopathic potential of *C. album* may be hypothesized due to the production and accumulation of phenolic compounds in significant amounts in the soil and resulting in phytoxicity to the neighbouring plants.

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