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Full Length Research Paper

Allelopathic potential of *Anagalis arvensis* L.

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Anagalis arvensis **L. (Primulaceae) is a common cultivating weed, forming dense populations of undergrowth in warm and temperate regions of Pakistan. Allelopathic studies with aqueous extracts from whole plant including leaves, flowers, shoot and roots; litter and mulch in various experiments invariably reduced the germination, plumule growth, radical growth, number of seminal roots, cell size, and fresh and dry weights of two wheat varieties namely, Ghaznavi and Uqab, which were used as the test species. Phytotoxicity of extracts depended on the amount and soaking duration. Generally, the extracts obtained after 48 h soaking and the hot water extracts were more inhibitory. Addition of litter and mulch also proved inhibitory in the experiments. Our work suggested that *A. arvensis* had strong allelopathic potential but must further be tested for its weedicidal and insecticidal activities. From the practical view point, the identification of weeds with potential pool of allelochemicals, screening and identification of the toxic principle, assessment of their adverse effects on germination of crops during early growth stages and finally on the commercial yield is highly recommended.**

Key words: *Anagalis arvensis* L. (Primulaceae), allelopathic, extracts, weed.

INTRODUCTION

Allelopathy is an interesting but complex mode of interaction between plants, accomplished through the release of chemical substances into the environment (Kruse et al., 2000; Willis, 2004; Bais et al., 2003; Machado, 2007). Scientists develop farming techniques, which are sustainable for the environment, crop production and protection as well as socio-economic point of view. Integrated weed management is one of such approach where allelopathy can be used as an eco-friendly tool in weed management and pathogens reduction (Hussain et al., 2007; Xaun et al., 2005; Dangwal et al., 2010).

Salam et al. (2011) found that *A. arvensis* significantly reduced mung radical growth while bajra radical growth was significantly enhanced by the root leachate. Dongre et al. (2004, 2010) reported that the aqueous leaf extracts of *Ageratum conyzoides*, *Anagallis arvensis*, *Chenopodium album*, *Parthenium hysterophorus* and *Rumex dentatus* significantly reduced various growth parameters that ultimately caused low yield of green gram (*Vigna radiata* Wilczek) var. K 851. *Anagallis* and *Senecio* have been shown to exert allelopathic effects on *Arabidopsis* (Bossdorf et al., 2009). Certain weeds including *Avena sterilis*, *Conzya canadense*, *Ammi majus*, *Datura stramonium, Cichorium endiia, Anagallis arvensis* and *Solanum nigrum* support *Orobanche ramose* attachment, but different resistant levels were observed and later led to the death of parasitic plant. These weeds may be used as trap species for *O. ramose* control (Boulet et al., 2001).

Anagallis arvensis inhibited germination, root and shoot growth of six test species due to the presence of salicylic acid, cinnamic acid and caffeic acid (Rebaz et al., 2001). Shoots of *A.arvensis* are found to have allelopathic effects against radish and lettuce seedlings (Alliotta et al., 1989). This review reveals that *A. arvensis* has novel allelopathic potentials. This present study is an addition to screening of allelopathic effects of *A. arvensis* against Ghaznavi and Uqab varieties of *Triticum aestivum.*

MATERIALS AND METHODS

A. arvensis was collected from the wheat fields of Azakhel Botanical garden, Nowshehra 34°0'55N 71°58'29E of Peshawar. They were

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dried at room temperature (25 to 30°C), powdered and stored in paper bags. Glassware, thoroughly washed with water, was sterilized at 170°C for at least 4 h. There were 5 replicates, each with 10 seeds. The Petri dishes were always incubated at 25°C for 72 h. All the results were statistically analyzed in one way ANOVA.

Effect of aqueous extracts

5 and 10 g of dried powdered shoots were soaked in 100 ml distilled water at 25°C for 24 and 48 h, respectively and filtered to get aqueous extracts. These extracts were tested against two varieties of wheat, that is *Triticum aestivum* Ghaznavi and Uqab on 3-folds of filter paper in Petri dishes. The filter papers were moistened with the respective extracts or the distilled water for making test and control. After 72 h germination, length of plumule, radicle and number of seminal roots were recorded. Twenty seedlings were randomly taken out from each treatment for fresh and dry weight determination. Seedlings were dried at 65°C for 72 h. The concentration of the aqueous extract was diluted to 50 and 25% with distilled water, in cases where no germination occurred at 5 g extract application.

Effect of hot water extracts

5 g of dried shoots were boiled in 100 ml water for 5 min and filtered. The room cooled extracts were applied against the same test species as before.

Effect of litter

5 g of crushed shoot litter was placed in Petri dish and topped with single sheet of filter paper. The dishes were provided with 5 ml distilled water. In the control treatment, fine pieces of filter paper were used to keep it moist throughout the bioassay. The bioassay was run as earlier explained.

Effect of mulching

5 g of crushed dried shoots were mixed with sterilized sand in plastic pots. There were five replicates, each with 10 seeds. Control consisted of fine pieces of filter paper. The plastic pots were incubated at 25°C. Germination, plumule and radical growth were measured after 15 days. Twenty seedlings were randomly taken out for the determination of fresh, dry weight and moisture contents. Moisture contents were determined on oven drying basis (Hussain, 1989).

Effect on cell size

The root tips saved from aqueous extract bioassay were placed in concentrated chloral hydrate solution. After 10 to 12 h, root tips were randomly taken out and pressed on a plain slide and examined under magnification. The sizes of the cells were measured at the tip from 3rd to 5th cortical layers. Five root tips were observed, each with 10 counts over a fixed distance in a row under microscope, following Hussain et al., (2009).

RESULTS AND DISCUSSION

Effect of aqueous extract

Aqueous extracts from shoots (Table 1) significantly reduced the germination, plumule and radical length of Ghaznavi variety at both concentrations especially at 10 g extract obtained after 48 h. Increasing soaking duration and concentration generally enhanced inhibition.

Similar to this present findings, *Dodonaea viscosa* (Barkatullah et al., 2010), *Lactuca sativa* (Chon et al., 2005), *Broussonetia papyrifera* (Hussain et al., 2004) and *Tamarindus indica* (Parvez et al., 2003) were also inhibitory in various bioassays. The findings of Rebaz et al. (2001) revealed that *A. arvensis* is allelopathic and this is similar to our results. The 5 g extract after 24 h was more inhibitory than the 48 h extract. This might be due to denaturation of some phytotoxic principles with the passage of time. Hussain et al. (2010, 2011) also stated *Cenchrus ciliaris* and *Bothriochloa pertusa* exhibited similar phytotoxicity. The phytotoxic effects were depending on variety. In this present study, it was seen that Uqab variety was completely inhibited even when treated with 5 g extract (Table 2) at both soaking durations that is 24 and 48 h. Likewise is the phytoxicity of sorghum + sunflower water extracts as reported by Mehmood et al. (2010). The 5 g/100ml extract at 24 h soaking was further diluted to 50 and 25% of the original strength which also showed significant inhibition at all tested concentrations. All the parameters except number of seminal roots were significantly decreased (seminal roots increased). Increasing of soaking duration and concentration generally enhanced inhibition. Many similar studies (Marwat and Azim, 2006; Hussain et al., 2007, 2011; Kamal and Bano, 2008; Hussain and Ilahi, 2009; Elizabeth et al., 2008; Barkatullah et al., 2010) agree with this present findings.

Fresh weight and dry weight of all test species also got retarded (Tables 1 and 2). These results agree with Kaul and Bansal (2002), who reported that *Ageratina adenphora* litter reduced growth of Lantana camara. Similarly, Maciel et al. (2003) also reached to similar conclusions. However, the moisture contents of seedlings increased as compared to the control. Some studies have reported decreased moisture contents (Pervez et al., 2003; Hussain et al., 2004; Hussain and Ilahi, 2009; Samreen et al., 2009; Barkatullah et al., 2010), thus contradicting this present findings but other workers like Fattah et al. (2011) have reported enhanced shoot moisture contents which are in line with this present findings. There are always divergent results when it comes to moisture contents. It is said that succulence might develop to counteract toxicity of extracts and this might be true in this present case.

Effect of hot water extract

Hot water extract from shoots significantly inhibited the germination and seedling growth of both test varities. However, the number of seminal roots significantly increased in case of Ghaznavi variety. It was also

Table 1. Effect of Aqueous extract of *Anagalis arvensis* on germination (%), plumule and radical growth (mm), number of seminal roots, fresh and dry weight (mg), and moisture contents (%) of variety Ghaznavi of wheat. Each value as a mean of five replicates, each with 10 seedlings.

Soaking duration and concentration	5 g Extract		10 g Extract	
	24 h	48 h	24 h	48 h
Germination (%)				
Control	100	100	100	100
Shoot extract	82**	80**	72**	56**
Plumule growth (mm)				
Control	32.76	32.76	31.56	31.56
Shoot extract	18.84	28.40	25.50	14.98**
Radical growth (mm)				
Control	29.46	29.46	29.56	29.56
Shoot extract	29.86	38.18	26.46	16.44*
Number of seminal roots				
Control	3.52	3.52	3.9	3.9
Shoot extract	5.14** (increase)	5.34** (increase)	3.06*	2.98*
Fresh weight (% of control)				
Shoot extract	46.87	35.71	87.15	51.37
Dry weight (% of control)				
Shoot extract	31.77	28.03	78.43	49.01
Moisture content (% of control)				
Shoot extract	43.48	152.42	120.93	109.05

*Significantly different from control at alpha 0.050 in one way ANOVA, **highly significantly different from control at alpha 0.010 in one way ANOVA.

observed that hot water extracts were more inhibitory than cold water extracts (Table 3). Chung et al. (2007), Peneva (2007), Hussain et al. (2004), Hussain and Ilahi (2009) and Barkatullah et al. (2010), also used hot water extracts and reported inhibitory effects against test species. Fresh weight, dry weight and moisture contents of tested plant seedlings generally reduced in various treatments. However, the inhibition was related to test varieties. It was interesting to see that moisture content got reduced by hot water extracts which is in contradiction to the findings of preceding experiment. It could be said that extraction through boiling and at room temperature might have had released different or different concentrations of allelochemicals. Although, the use of hot water extract is unnatural yet it not only reduces the time period for extraction of allelochemicals but phytotoxins retain their phytotoxicity.

Effect of litter

In nature, litter from crops/plants is used as organic matter with the assumption that it promotes growth of crops. However, it was observed that when litter is used as a growth medium, it significantly reduced the germination, radical and plumule growth of both test varieties. However, the number of seminal roots of Ghaznavi variety significantly increased. Fresh and dry weight of both species also got retarded (Table 3). These results agree with Kaul and Bansal (2002), who reported that *Ageratina adenphora* litter reduced growth of *Lantana camara*. Similarly, Maciel et al. (2003) also reached similar results. Litter from *Cenchrus ciliaris* and *Bothriochloa pertusa* (Hussain and Ilahi, 2009; Hussain et al., 2010, 2011) proved inhibitory to test species. Fresh weight, dry weight and moisture content showed a similar trend as in case of aqueous extracts.

Effect of mulching

Allelopathic substances released by the plants are accumulated in the soil to physiological activity level (Hussain et al., 2004; Hussain and Ilahi, 2009; Samreen

Table 2. Effect of aqueous extract of *Anagalis arvensis* on germination (%), plumule and radical growth (mm), number of seminal roots, fresh and dry weight (mg), and moisture contents (%) of variety Uqab of wheat. Each value as a mean of five replicates, each with 10 seedlings.

Soaking duration and concentration	5 g (100%)		50%		25%	
	24 h	48 h	24 h	48 h	24 h	48 h
Germination (%)						
Control	100	100	100	100	100	100
Shoot extract	0	0	100	74**	100	86**
Plumule growth (mm)						
Control	34.66	34.66	34.66	34.66	34.66	34.66
Shoot extract	0	0	23.40**	5.76**	32.64	10.56**
Radical growth (mm)						
Control	45.04	45.04	45.04	45.04	45.04	45.04
Shoot extract	0	0	20.76**	16.96**	26.80**	20.36**
Number of seminal roots						
Control	2.64	2.64	2.64	2.64	2.64	2.64
Shoot extract	0	0	3.28*(increase)	2.34	3.92** (increase)	3.14* (increase)
Fresh weight (% of control)						
Shoot extract	0	0	49.11	13.53	56.47	19.70
Dry weight (% of control)						
Shoot extract	0	0	73.75	37.50	78.75	40.00
Moisture content (% of control)						
Shoot extract	0	0	56.31	16.4	63.01	33.63

*Significantly different from control at alpha 0.050 in one way ANOVA, **highly significantly different from control at alpha 0.010 in one way ANOVA.

et al., 2009). Inderjit and Duke (2003) stated that plants release phytochemicals from dead tissues, and their incorporation to the soil could be accelerated by leaching, thus facilitating their harmful effects in the field. This aspect when tested, by using shoot mulch in experiments, significantly inhibited test varieties. The germination, plumule and radical growth and number of seminal roots in both test varieties were retarded. Maximum inhibited germination was in Uqab variety, while maximum reduction of radical growth occurred in Ghaznavi variety. Fresh weight and dry weight of all test species also got retarded (Table 4). These findings agree with those of Rebaz et al. (2001), Hussain et al. (2004), Eppard et al. (2005) and Barkatullah et al. (2010) who also observed that litter and mulches were phytotoxic to other plants. However, the moisture contents once again increased as in previous aqueous extract bioassay.

Effect on cell size

Effect of 5 g, 24 and 48h cell size of Ghaznavi

Table 3. Effect of hot water extract and litter of *Anagalis arvensis* on germination (%), plumule and radical growth (mm), No of seminal roots, Fresh and dry weight (mg), and moisture contents (%) of variety Ghaznavi and Uqab of wheat. Each value is a mean of five replicates, each with 10 seedlings.

Treatment	Litter		Hot water extract	
	Ghaznavi	Uqab	Ghaznavi	Uqab
Germination (%)				
Control	100	100	100	100
Shoot extract	90*	48**	90*	94
Plumule growth (mm)				
Control	32.76	47.18	32.76	47.18
Shoot extract	18.6**	4.96**	10.5**	4.68**
Radical growth (mm)				
Control	29.48	48.70	29.48	48.70
Shoot extract	22.38	9.2**	16.74*	17.62**
Number of seminal roots				
Control	3.52	3.76	3.52	3.76
Shoot extract	4.94** (increase)	2.48**	4.98** (increase)	2.92**
Fresh weight (% of control)				
Shoot extract	34.82	45.31	52.68	76.56
Dry weight (% of control)				
Shoot extract	30.84	62.50	63.55	75.00
Moisture content (% of control)				
Shoot extract	124.70	51.09	67.24	103.65

*Significantly different from control at alpha 0.050 in one way ANOVA, ** Highly significantly different from control at alpha 0.010 in one way ANOVA.

Table 4. Effect of shoot Mulch of *Anagalis arvensis* on germination (%), plumule and radical growth (mm), number of seminal roots, Fresh and dry weight (mg), and moisture contents (%) of variety Ghaznavi and Uqab of wheat. Each value as a mean of five replicates, each with 10 seedlings.

Treatment	Ghaznavi	Uqab
Germination (%)		
Control	82	100
Intoxicated soil	64	18**
Plumule growth (mm)		
Control	149.00	80.72
Intoxicated soil	152.16	67.18
Radical growth (mm)		
Control	62.00	73.42
Intoxicated soil	42.06**	67.22
Number of seminal roots		
Control	3.66	3.7
Intoxicated soil	3.64	2.52

Table 4. Continue

Number of Leaves		
Control	2	2
Intoxicated soil	1.88	1.4
Fresh weight (% of control)		
Intoxicated soil	79.46	25.14
Dry weight (% of control)		
Intoxicated soil	43.96	29.62
Moisture content (% of control)		
Intoxicated soil	138.87	80.35

*Significantly different from control at alpha 0.050 in one way ANOVA, **highly significantly different from control at alpha 0.010 in one way ANOVA.

eat variety. It was observed that the cell size was significantly reduced at all concentrations. The mean cell size in control measured 18.62 µ, while it measured 8.51 and 14.24 µ in case of 24 and 48 h extract, respectively. Seedlings of Uqab variety treated with 50 and 25% of 5 g, 48 h extract also showed significant reduction in cell size. Where the mean cell size in control was 14.34 µ and reduced to 9.696 and 6.528 µ by 25 and 50% extract, respectively. All these values differed significantly from that of the control conditions. The concentrated extract was more inhibitory. Our results are supported by the work of Hussain and Ilahi (2009) and Nishida et al. (2005), who reported reduction in cell division and cell size due to allelopathy. However, 24 h extract was more inhibitory than 48 h extract. This result is contradictory to the findings of Hussain et al. (2009).This might be due to denaturation of some phytotoxic principles with the passage of time.

The mode of action of allelochemicals spans over a wide range of actions including lack of cell division, cell development, cell lysis, blistering or growth inhibition (Wu et al*.,* 2003). *A. arvensis* has been reported to contain oleanane triterpene **(**Aliotta et al*.,* 1992), three phenolic acids that is salicylic acid, cinnamic acid and caffeic acid (Rebaz et al., 2001), flavonoids and saponin (Mojab et al., 2003). All these substances might be responsible for its allelopathy.

This present study suggests that *A. arvensis* is allelopathic plant, which is capable of suppressing the germination and growth of Wheat crop. Allelophathic effects depended upon the variety and physiological process involved. These results show that in all the bioassays, Uqab variety was more sensitive than Ghaznavi variety. Although, this present results are laboratory based, yet it indicates the capability of *A. arvensis* to release allelopathic substances through water. In nature, it is quite possible that the *A. arvensis* is one of the causes for reduction in our wheat yields, due to its allelopathy. However, further study is needed to explain allelopathic mechanism and to identify the allelopathic principle. It may also be investigated to test its efficacy as a weeds, pests and disease control agent.

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