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Full Length Research Paper

Allelopathic effects of *Rhazya stricta* decne on seed germination and seedling growth of maize

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This study was designed to investigate the allelopathic potential of *Rhazya stricta* leaves and stem on *Zea mays.* It showed that stem of aqueous extracts of *R. stricta* present inhibitory effects on germination and seminal root numbers, while leave extract significantly decreased the plumule and radical growth of test specie. The inhibitory effects were increased proportionally with increasing extract concentration. These findings indicate that both germination and growth *of Z. mays* sown in fields, which had leaf and stem litter of *R. stricta* will be adversely affected, ultimately resulting to lower yield.

Key words: *Rhazya stricta,* allelopathy, competition, aqueous leaf extract, aqueous stem extract.

INTRODUCTION

Allelopathic studies were undertaken earlier (Schreiner and Reed, 1907, 1908) while one of the defining experiments in the history of allelopathy was carried out by Massey (1925). Simply allelopathy is chemical competition between plants (Ricklefs, 1990). The definition is sometimes broadened to include non-antagonistic relationships between organisms (Whittaker and Feeny, 1971). In agroecosystems, allelopathic effects between living weeds and crops, crops in mixtures, plant straw residue and succeeding crops during decomposition of residue are also well documented (Rice, 1984). Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition, and other processes in both natural and agricultural systems (Gibson and Liebman, 2003). Allelopathy is expected to be an important mechanism in the plant invasion process because of the lack of co-evolved tolerance of resistant vegetation to new chemicals produced by the invader. This phenomenon could allow the new introduced species to overlook natural plant communities. Allelopathy is the production and release of chemicals that harm or otherwise decrease the fitness of other plants (Hierro and Callaway, 2003). Allelopathy was also studied by many researchers (Torneau and Heggenesse, 1957; Muller, 1966; Kaminsky, 1981; Muir and Majak, 1983; Weston et al., 1987; Ricklefs, 1990; Pandey, 1994; Ohno, 2001; Bais et al., 2002; Alford et al., 2009; He et al., 2009; Thorpe et al., 2009).

Rhazya stricta Decne., an evergreen poisonous under shrub, has covered large small hill of District Karak, Pakistan. *R. stricta* like other plants is competing with the main crops for nutrients and other resources and hamper the healthy growth of crops ultimately, reducing the yield both qualitatively and quantitatively. Ahmad et al. *(*1983) and Al-Yahya et al. *(*1990) have reported the presences of alkaloids, glycosides, triterpenes, tannins and volatile bases in the leaves of this plant. To explore allelopathic potential of *R. stricta* we examined the effect of aqueous extract of leaves and stem of this plant on seed germination and seedling growth of *Zea mays* species which come with rain water to the field and growing naturally together with *R. stricta*.

MATERIALS AND METHODS

Collection of plant materials

Plant of *R. stricta* Decne was collected from District Karak, Khyber

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Table 1A. Allelopathetic effects of *Rhazya stricta* on germination counts of maize.

Extract	Duration	24 h		48 h		Means
	Concentrations	5 g	10 g	5 g	10 g	
Control		100	100	100	100	100
Leave		96	100	100	96	98
Stem		96	96	92	92	94*
		97	99	97	96	
		98		97		

Table 1B. Analysis of variance on germination counts of maize.

K value	Source	Degrees of freedom (DF)	Sum of squares	Mean square	F value	Probability
1	Replication	4	40	10	0.2	
2	Factor A	2	373.333	186.667	3.7333	0.0318
4	Factor B	1	26.667	26.667	0.5333	
6	AB	2	53.333	26.667	0.5333	
8	Factor C	1	0	0	0	
10	AC	2	0	0	0	
12	BC	1	26.667	26.667	0.5333	
14	ABC	2	53.333	26.667	0.5333	
-15	Error	44	2200	50		
	Total	59	2773.333			

Factor A: extract; factor B: duration; factor C: concentration.

Pukhtoonkhwa, Pakistan. Plants were then washed thoroughly with water and dried in open air under natural condition. Leaf and stem samples were separately powdered and stored in plastic bottles at room temperature.

Preparation of aqueous extract

Five and ten grams powdered leaves and stems soaked in 100 ml distilled water for 24 h at room temperature. Aqueous extract were filtrated and final volume was adjusted to 100 ml. The extract was considered as stock solution.

Treatments and experimental design

Ten healthy and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *Z. mays* were kept for germination in sterilized petri-dishes on 2-folds of blotting paper and moistened with 10 ml extracts. Each treatment had 5 replicates each with 10 seeds. Control consisted of distilled water. The petri-dishes were maintained under laboratory conditions at 25°C temperature with diffused light during day.

Physical parameters

After seven days, germination was counted and the length of root and shoot were measured and number of seminal roots was counted.

Statistical analysis

The data obtained was subjected to three way analysis of variance, randomized complete block design (RCBD) and the mean values were separated at $P < 0.05$ applying least significant difference test (LSD).

RESULTS

Effect on germination

Leaf extract did not inhibit germination while stem extract of both concentrations at 48 h inhibited germination. ANOVA (RCBD) (DF 1, 44) showed significant effects of stems extracts (F=3.7333) on germination, while the effect of treatment duration and concentration was not significant (Table 1A and B).

Effect on plumule growth

Leaf extract of all the treatments significantly reduced the plumule growth (Table 2A), while in the case of stem extracts only 10 g extract after 48 h was inhibitory. ANOVA (RCBD) (DF 1, 44) showed significant inhibitory effects of leaves (F = 59.7), especially at 48 h treatment (F = 10.8) and 10 g concentration (F = 13.8) on plumule length. Comparison of extract and duration (F = 6.6775) and extracts and concentration (F = 5.0755) showed a significant differences of 48 h treatment and 10 g concentration of leave extract, respectively. Comparison

Table 2A. Allelopathetic effects of *Rhazya stricta* on plumule length of maize.

Extract	Duration	24 h		48 h		Mean			
	Concentrations	5 g	10 g	5 g	10 g	M1	M2	M3	M4
Control		69.1	69.1	69.1	69.1	69.1	69.1	69.1	
Leave		31.1	15.6	23.2	11.8	20.5*	17+	13.7+	
Stem		92.6	67.4	65.0	28.4	63.4	46.7	47.9	
		64.3	50.7	52.4	36.4				43.55*
		57.5		44.4*					

M1: Mean of all extracts; M2 = mean of 48 h (5 +10 g) leave extract; M3 = mean of 10 g (24 + 48 h) leave extract; M4 = mean of 10 g (24 + 48 h), *: within groups, +: between groups.

Table 2B. Analysis of variance on plumule length of maize.

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
1	Replication	4	2369.061	592.265	2.5027	0.0559
2	Factor A	2	28250.507	14125.254	59.6884	0
4	Factor B	1	2550.624	2550.624	10.778	0.002
6	AB	2	3160.441	1580.221	6.6775	0.0029
8	Factor C	1	3276.726	3276.726	13.8463	0.0006
10	AC	2	2402.239	1201.119	5.0755	0.0104
12	BC	1	22.571	22.571	0.0954	
14	ABC	2	161.054	80.527	0.3403	
-15	Error	44	10412.595	236.65		
	Total	59	52605.819			

Table 3A. Allelopathetic effects of *Rhazya stricta* on radical length of maize.

Extract	Duration	24 h		48 h		Means			
	Concentrations	5 g	10 g	5 g	10 g	M1	M2	M3	M4
Control		72.97	72.97	72.97	72.97	72.97	72.97	72.97	
Leave		16.68	9.40	24.91	7.62	14.65*	13.04+	8.51+	
Stem		127.98	84.88	90.96	27.37	82.80	106.43	56.12	
		72.54	55.75	62.94	35.99				45.87*
		64.15		49.47*					

M1: Mean of all extracts; M2 = 24 h (5 + 10 g) leave extract; M3 = 10 g (24 and 48 h) leave extract; M4 = 10 g (24 and 48 h) all extract.

of extracts, duration and concentration and comparison of concentration and duration was found insignificant (Table 2A and B).

Effect on radical growth

The radical growth was significantly reduced by leaf extracts in all treatments, especially in concentration and 48 h extracts (Table 3). All the extracts from stems except 10 g in 48 h stimulate radical growth. ANOVA (RCBD) (DF 1, 44) showed significant effects of leaves (F=56.4604), 48 h treatment (F = 6.7273) and 10 g concentration (F = 14.9350) on radical length. Comparison of extract and duration (F = 8.3111) and extracts and concentration (F = 8.1179) showed significant inhibitory effect of 48 h treatment and 10 g concentration of leave extract respectively (Table 3A and B).

Effect on number of seminal roots

There was slight reduction in number of seminal roots in all treatment of leaves while stem extracts with few exceptions were generally stimulatory (Table 4). ANOVA (RCBD) (DF 1, 44) showed significant inhibitory effects of stem (F=9.0556) on numbers of seminal roots of maize. Comparison of extracts and concentration (F = 4.4675)

Table 3B. Analysis of variance on radical length of maize.

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
1	Replication	4	1862.91	465.728	0.969	
2	Factor A	2	54274.504	27137.252	56.4604	0
4	Factor B	1	3233.417	3233.417	6.7273	0.0128
6	AB	2	7989.304	3994.652	8.3111	0.0009
8	Factor C	1	7178.39	7178.39	14.935	0.0004
10	AC	2	7803.593	3901.797	8.1179	0.001
12	BC	1	387.401	387.401	0.806	
14	ABC	2	262.24	131.12	0.2728	
-15	Error	44	21148.253	480.642		
	Total	59	104140.012			

Table 4A. Allelopathetic effects of *Rhazya stricta* on seminal root numbers of maize.

Extract	Duration	24 h		48 h		Means	
	Concentrations	5 g	10 g	5 g	10 g	M1	M2
Control		6.2	6.2	6.2	6.2	6.2	6.2
Leave		5.7	4.3	6.0	5.2	5.3	5.85
Stem		5.0	4.4	4.0	6.1	4.9*	4.5+
		5.6	4.9+	5.4	5.8		
		5.3		5.6			

M1: Mean of all extracts, M2 = 5 g (24 and 48 h) stem extract.

Table 4B. Analysis of variance on seminal root numbers counts of maize.

K value	Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
1	Replication	4	7.284	1.821	1.9368	0.1211
2	Factor A	2	17.029	8.515	9.0556	0.0005
4	Factor B	1	1.601	1.601	1.7024	0.1988
6	AB	2	0.881	0.441	0.4687	
8	Factor C	1	0.241	0.241	0.256	
10	AC	2	8.401	4.201	4.4675	0.0171
12	BC	1	4.483	4.483	4.7674	0.0344
14	ABC	2	5.157	2.579	2.7425	0.0754
-15	Error	44	41.372	0.94		
	Total	59	86.449			

showed that there was a significant reduction of 5 g concentration of stem extract on numbers seminal roots of maize and concentration and duration (F= 4.7674) showed significant differences of 10 g in 24 h treatment (Table 4A and B).

DISCUSSION

The present study suggested the presence of various allelochemical in aqueous extract from leaves and stem which exhibited allelopathic stress against the germination, seedling growth, and number of seminal roots of tested specie. Aqueous extracts from leave and stem parts significantly reduced the germination, plumule and radical growth and number of seminal roots of test specie. The extract from leaves had more inhibitory effects than stem (Tables 1 to 4). The aqueous extracts from stem stimulated the seedling growth of maize. Plumule and radicle growth of maize were observed to be more sensitive than the others (Tables 1 to 4). Aqueous extracts from leaves obtained after 48 h had more

inhibitory effects than 24 h extracts (Tables 1 to 4). Increasing soaking duration and concentration generally enhanced inhibition. Similarly, the phytotoxicity of *Azadirachta indica* (Xuan et al., 2004), *Tamarindus indica* (Parvez et al., 2003), *Broussonetia papyrifera* (Hussain et al., 2004) and *Lactuca sativa* (Chon et al., 2005) is generally enhanced with soaking duration, and this supports our findings. Marwat and Azim (2006), Hussain et al. (2007) and Elizabeth et al. (2008) also reported similar phytotoxicity for other plant species, which agree with our present results.

In this study, allelopathic effects of *R.stricta* were observed on germination and seedling growth of *Z. mays*. It is evident from the result that stem aqueous extracts of *R. stricta* exhibited more inhibitory effects on germination and seminal root numbers of test specie while leave extract present inhibitory effect on plumule length and radical length as compared to control (Tables 1 to 4). The results of our study showed that the leaf and stem extracts of *R. stricta* present inhibitory effect in maize. Similar results have been reported by many researchers (Hussain and Gadoon, 1981; Worsham, 1984; Aslam and Azmi, 1989) while studying the allelopathic effect of different plants. They observed that the foliar leachates have been more phytotoxic in nature.

Comparative analysis between extracts and duration showed significant inhibitory effect of 48 h treatment on plumule and radical length. Furthermore, the comparison of duration and concentration showed a significant inhibitory effect of 10 g concentration in 24 h treatment on the seminal root number, and the comparison of extracts and concentration gave a significant inhibitory effect of 10 g leave extract on plumule and radical length while 5 g stem extract had effect on the seminal root number. The result shows that the inhibitory effects were increased proportionally with the increase in extract concentrations and duration. The present findings corroborate the earlier results reported by Bora et al. (1999) who found out that, the inhibitory effect of leaf extracts of *Acacia auriculiformis* on the germination of some agricultural crops was proportional to the concentration of the extract. Also, as noted by Jadhar and Gayanar (1992) the percentage of germination, plumule and radicle length of rice and cowpea, were decreased with increasing concentration of *A. auriculiformis* leaf leachates. Several studies documented the allelopathic effect of various plants that significantly affected seed germination and seedling growth of several crops and weed species (Khan et al., 2004; Lisanework and Michelson, 1993), and these studies showed that the leaf extract of *E. camaldulmensis* decreased root growth of the majority of the crops. Similar findings were also reported by many researchers (Tefera, 2002; khan et al., 2003; Siddiqui et al., 2009) in leaf extract of different trees in common agricultural crops. They found an inhibitory effect in seed germination and radicle length and other initial parameters.

Plant litter generally increases soil fertility during decay but it has been seen that many species release phytotoxic substances before decay. It was observed that litter from leaves and stem when used as growth medium significantly reduced the germination, radical and plumule growth and number of seminal roots of test specie. 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 results.

Allelopathic substances released by the plants accumulate in the soil to physiologically active levels (Hussain et al., 2004). Inderjit and Duke (2003) stated that plants release photochemicals 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 *R. stricta* in experiments significantly inhibited test species. These findings agree with those of Hussain et al. (2004) who also observed similar phytotoxicity by other plants.

Its effectiveness on germination and growth suggests that leaves and stem of *R. stricta* can act as a source of allelochemicals after decomposition that in-turn negatively affects the neighboring or successional plants. The observed different phytotoxicity of *R. stricta* may be attributed to the presence of variable amount of phytotoxic substances in different parts that leach out under natural conditions. Some recent studies indicate the phytotoxic/allelopathic effect of aqueous extracts of weeds which include *Parthenium hysterophorus* (Singh et al., 2003) and *Ageratum conyzoides* (Batish et al., 2002). All these studies strongly showed the release of phototoxic chemicals during the preparation of aqueous extracts.

Conclusions

The present investigation revealed that *R. stricta* leaves and stem extracts contain some substances which have inhibitory effects on *Z. mays*. Therefore, further studies are recommended to investigate the possible physiological mechanisms related to allelopathic effect on plants.

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