



Allelopathic potential of *Rhazya stricta* Decne on germination of *Pennisetum typhoides*

Musharaf Khan^{1*}, Farrukh Hussain¹, Shahana Musharaf²

¹Department of Botany, University of Peshawar, Pakistan

²Government Girls Degree College Sheikh Malton Mardan, Pakistan

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Abstract

Rhazya stricta Decne. an evergreen poisonous shrub of the Apocynaceae family locally known as Ganderi, is a wild plant widely distributed in the hilly area of District Karak Pakistan and comparable habitats throughout the world. This study was designed to investigate the allelopathic potential of *R. stricta* (stem and leaves) on *Pennisetum typhoides*. Results showed that 10g aqueous extracts of leaves and 48 hours treatment present inhibitory effect on germination percentage, radical length and seminal root number and the effect was found significantly higher than that recorded in the stem and control treatment. The inhibitory effects were increased proportionally with the extract concentration and treatment duration. These findings indicate that *P. typhoides* sown in fields which had leaf and stem litter of *R. stricta* will be adversely affected regarding germination, growth and ultimately resulting in lower yields of *P. typhoides*.

*Corresponding Author: Musharaf Khan ✉ k.musharaf@gmail.com

Introduction

Plants live in association in groups depending upon the ecological requirements; they have generally the same structural and morphological adaptations. Whenever two or more plants occupy the same niche in nature, they compete with each other for various life support requirements (Caton *et al.*, 1999). Rice in 1984 defined Allelopathy as the effects of one plant (including microorganisms) on another plant via the release of chemicals into the environments. Allelopathy regards these effects due to chemicals released by them, or the breakdown products of their metabolites (Willis, 1994). Allelopathy has been suggested as a mechanism for the impressive success of invasive plants by establishing virtual monoculture and may contribute to the ability of particular exotic species to become dominants in invaded plant communities (Hierro, 2003; Kanchan and Jayachandra, 1979). Allelopathy is expected to be an important mechanism in the plant invasion process because 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 (Hierro, 2003). In fact, allelopathic interference is one of the important mechanisms for the successful establishment of invasive exotic weeds (Ridenour & Callaway, 2001).

Rhazya stricta Decne., an evergreen poisonous shrub, has covered large hilly areas of District Karak, Pakistan. *Rhazya stricta* like other weeds compete with the main crops for nutrients and other resources and hamper the healthy growth 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 effect of aqueous extract of leaves and stem of this plant on seed germination and seedling growth of *P. typhoides* specie growing naturally together with *R. stricta*.

Materials and methods

Collection of plant materials

Plants of *R. stricta* were collected from District Karak, Khyber Pakhtonkhwa, Pakistan. Plants tissues were washed several time with water and dried in open air and under natural light. Leaf and stem samples were grounded and the powdered material were stored in plastic bottles at room temperature.

Preparation of aqueous extract

Five and ten gram of air dried leaves and stem of *R. stricta* were grounded, mixed with 100 ml distilled water and left for 24 hr at the room temperature (average during day: 25°C) in dark conditions . Aqueous extract was obtained as filtrate of the mixture and final volume was adjusted to 100 ml; this gave 5 and 10g aqueous extract. The extract was considered as stock solution.

Treatments and experimental design

Ten uniform and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *P. typhoides* were kept for germination in sterilized petri-dishes lined double with blotting paper and moistened with 10 mL of 5 and 10g concentrations of aqueous extracts. Each treatment had five replicates (total number of test seeds: 10 x 5 = 50). One treatment was run as control with distilled water only. The petri-dishes were maintained under laboratory conditions (room temperature 25°C at mid day, and diffused light during day). The whole experiment was repeated once.

Physical parameters

After seven days, the seedling root length (cm), shoot length (cm) were measured while number of germinated seeds and seminal root number were 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

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves ($F=24.7483$, $P < 0.05$) on germination. Comparison of extracts, duration and concentration showed significant effects of 10g concentration of leaves in 24hr treatment ($F=10.6142$, $P < 0.05$). (Table 1a and 1b).

Table 1a. Allelopathetic effects of *R. stricta* on germination of *P. typhoides*.

Extract	Duration		24hr		48hr		Means	
	Concentration		5g	10g	5g	10g		
Control			100	100	100	100	100	
Leave			80	62+	78	84	76*	
Stem			78	90	96	68	83	
			86	84	91.33	84		
			85		87.66			

*: within group + : between group

Table 1b. Analysis of variance on germination (%) of *P. typhoides*.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	143.33	35.833	0.2911	
2	Factor A	2	6093.33	3046.66	24.748	0
4	Factor B	1	106.667	106.667	0.8665	
6	AB	2	413.333	206.667	1.6788	0.1983
8	Factor C	1	326.667	326.667	2.6535	0.1105
10	AC	2	173.333	86.667	0.704	
12	BC	1	106.667	106.667	0.8665	
14	ABC	2	2613.333	1306.667	10.6142	0.0002
-15	Error	44	5416.667	123.106		
	Total	59	15393.33			

Factor A: Extract, Factor B: Duration, Factor C: Concentration

Effect on plumule growth

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves ($F=239.0506$, $P < 0.05$), and 10g concentration ($F=30.1707$, $P < 0.05$) on Plumule length. Comparison of extract (Stem and leave of *R. stricta*) and duration ($F=20.2093$, $P < 0.05$) and extracts and concentration ($F=7.7343$, $P <$

0.05) showed significant effect of 24 hr treatment and 10 gram concentration of leaves extract respectively. In comparison of concentration and duration 10g concentration in 48hr showed significant effect ($F=10.0136$, $P > 0.05$) and comparison of extracts, duration and concentration showed significant effects of 10g concentration of leaves extract in 24hr treatment ($F=10.8186$, $P < 0.05$). (Table 2a and 2b).

Table 2a. Allelopathetic effects of *R. stricta* on plumule length of *P. typhoides*.

Extract	Duration		24hr		48hr		Means	
	Conc.		5g	10g	5g	10g	M1	M2
Control			51.2	51.2	51.2	51.2	51.2	
Leave			18.1	5.44+	24.4	12.4	15.1*	11.7+
Stem			44.4	47.5	43.5	19.9	51.7	45.9
			M5	37.9	34.7	39.7	27.8+	31.2*
				36.3		33.7		

M1= Mean of each extract (5 & 10g), M2= Mean of 24h of 5g+10g Leaves extract, M3= Mean of 10g in 24 & 48hr, M4= Mean of 10g leaves extract in 24 & 48hr, M5= Mean of 10g in 48hr.

Table 2b. Analysis of variance on plumule length of *P. typhoides*.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	146.70	36.676	1.2999	0.2848
2	Factor A	2	13489.64	6744.82	239.05	0
4	Factor B	1	96.774	96.774	3.4299	0.0707
6	AB	2	1140.41	570.20	20.209	0
8	Factor C	1	851.267	851.267	30.1707	0
10	AC	2	436.449	218.225	7.7343	0.0013
12	BC	1	282.534	282.534	10.013	0.0028
14	ABC	2	610.492	305.246	10.818	0.0002
-15	Error	44	1241.46	28.215		
	Total	59	18295.74			

Factor A: Extract, Factor B: Duration, Factor C: Concentration

Effect on radical growth

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves ($F=204.7997$, $P < 0.05$) on radical length. Comparison of duration and

concentration ($F= 4.7034$, $P< 0.05$) showed significant effect of 48 hr treatment and 10 gram concentration of extract. Comparison of extracts, duration and concentration showed significant effects of 10g concentration of leaves in 24 hr treatment ($F= 4.5068$, $P< 0.05$). (Table 3a and 3b).

Table 3a. Allelopathetic effects of *R. stricta* on radical length of *P. typhoides*.

Extract	Duration 24hr		48hr		Means	
	Conc.	5g	10g	5g		10g
Control		80.08	80.08	80.08	80.08	
Leaf		4.29	2.27+	6.68	4.08	4.33*
Stem		28.96	36.97	36.13	3.84	26.47
		37.77	39.77	40.96	29.33+	
		38.77		35.14		

Table 3b. Analysis of variance on radical length of *P. typhoides*.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	449.251	112.313	0.7582	
2	Factor A	2	60675.002	30337.50	204.79	0
4	Factor B	1	197.146	197.146	1.3309	0.2549
6	AB	2	667.541	333.771	2.2532	0.1171
8	Factor C	1	347.908	347.908	2.3486	0.1326
10	AC	2	415.526	207.763	1.4025	0.2567
12	BC	1	696.732	696.732	4.7034	0.0355
14	ABC	2	1335.209	667.605	4.5068	0.0166
-15	Error	44	6517.831	148.133		
	Total	59	71302.14			

Factor A: Extract, Factor B: Duration, Factor C: Concentration

Effect on number of seminal roots

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves ($F=400.9321$, $P< 0.05$) and 48h treatment ($F=23.3889$, $P< 0.05$) on seminal root number. Comparison of extract and duration showed significant effects of leaves extract in 24 hr treatment ($F=33.8362$, $P< 0.05$) (Table. 4a and 4b).

Discussion

In the present study allelopathetic effects of *R. stricta* was observed on germination and seedling growth of *P. typhoides*. From result it was found that leaf and stem extract had the strongest allelopathetic effect on seed germination. The study demonstrated that

leaves aqueous extracts of *R. stricta* exhibited significant inhibitory effects on seed germination and seedling growth of test specie. This indicates the availability of the inhibitory chemicals in higher concentration in leaves than in stem. Tefera 2002 also reported that foliar leachates have been more phytotoxic in nature.

Table 4a. Allelopathetic effects of *R. stricta* on numbers of seminal roots of *P. typhoides*.

Extract	Duration 24hr		48hr		Means		
	Conc.	5g	10g	5g	10g	M1	M2
Control		2.52	2.52	2.52	2.52	2.52	2.52
Leaf		0.80	0.62	0.78	0.84	0.76	0.71+
Stem		1.74	1.62	0.96	0.68	1.25	1.68
		1.69	1.59	1.42	1.35		
		1.64		1.38 *			

M1= Mean of each extract (5 & 10g), M2= Mean of 5g & 10g in 24h.

Table 4b. Analysis of variance on numbers of seminal roots of *P. typhoides*.

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	4	0.069	0.017	0.4191	
2	Factor A	2	33.004	16.502	400.93	0
4	Factor B	1	0.963	0.963	23.388	0
6	AB	2	2.785	1.393	33.836	0
8	Factor C	1	0.113	0.113	2.7373	0.105
10	AC	2	0.105	0.053	1.2796	0.288
12	BC	1	0.003	0.003	0.0648	
14	ABC	2	0.101	0.051	1.231	0.301
-15	Error	44	1.811	0.041		
	Total	59	38.95			

Factor A: Extract, Factor B: Duration, Factor C: Concentration

The present investigation revealed that aqueous extract of *R. stricta* at various concentration levels inhibited the germination percentage, radical length, Plumule length and seminal root numbers. The comparative analysis between germination percentage and extract concentration showed that 48h treatment of 10g leaves extract have produced more inhibitory effect on germination of *P. typhoides*. Its effectiveness on germination and growth suggests that leaves and stem of *R. stricta* may act as a source of allelochemicals after being released into soil or after decomposition that intern

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 indicating the phytotoxic/ allelopathic effect of aqueous extracts of weeds include *Parthenium hysterophorus* (Singh *et al.*, 2003), *Brassica nigra* (Tawaha and Turk, 2003), *Raphanus raphanistrum* (Norsworthy, 2003) and *Ageratum conyzoides* (Batish *et al.*, 2002). All these studies indicate the release of phototoxic chemicals during the preparation of aqueous extracts. Based on this, studies were further extended to explore the impact of *R. stricta* (especially) leaves, as they possessed greater phytotoxicity on the emergence and growth of weed plants.

Conclusion

The present investigation revealed that its effectiveness on germination and growth suggests that leaves of *R. stricta* may act as a source of allelochemicals after being released into soil or after decomposition. The presence of allelochemicals negatively affects the neighboring or successional plants. Further studies are suggested to clarify the possible physiological mechanism related to allelopathic effect on plants.

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