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Full Length Research Paper

Inhibitive effects of *Chrozophora obliqua* (del.) juss. on germination and seedling growth of cultivated species

Musharaf Khan[1]* and Shahana Musharaf[2]

[1]Department of Botany, University of Peshawar, Pakistan.
[2]Department of Chemistry, Government Girls Degree College S. Malton, Mardan, Pakistan.

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The allelopathic influence of aqueous extracts of *Chrozophora obliqua* on the germination and seedling growth of *Pennisetum typhoides* (Bajra) have been determined. It was eminent that 10 g aqueous extracts of leaves and 48 h treatment present inhibitory effect on germination percentage, radical length and number of seminal roots and the cause was found significantly higher than that recorded in the stem and control treatment. The inhibitory effects were increased proportionally with increasing extract concentration and soaking duration. The only exception was observed in the 5 g concentration of leaves that decreased the plumule length. These findings indicate that *P. typhoides* sown in fields which had leaf and stem litter of *C. obliqua* will be adversely affected regarding germination, growth and ultimately resulting in lower yield.

Key words: Allelopathy, aqueous extracts, *Chrozophora oblique*.

INTRODUCTION

The term allelopathy was introduced by Molisch in 1937 and is derived from the Greek words allelon ‘of each other’ and pathos ‘to suffer’ and mean the injurious effect of one species upon the other (Rizvi et al., 1992). Rice (1984), defined allelopathy as the effect 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 and Callaway, 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 and Callaway, 2003). In fact, allelopathic interference is one of the important mechanisms for the successful establishment of invasive exotic weeds (Ridenour and Callaway, 2001).

Chrozophora obliqua (Del.) Juss., Euphorbiaceae, locally known as Harassina, grows in various parts of Pakistan. To explore allelopathic potential of *C. obliqua* 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 *C. obliqua*. Like elsewhere in Pakistan, weeds pose a serious problem in crop production. Because of lack of education and financial resources, the smaller farmers cannot afford to remove them from their fields. Weeds growing among crop plants adversely affect yield and quality of the harvest and increase production costs, resulting in high economic losses (Alam, 1991). Weeds are undesirable plants. Plants which interfere with human activity in crop and non-crop areas are considered as weed (Anon, 1994). They compete with the main crops for nutrients and other resources and hamper the healthy growth ultimately, reducing succumb both qualitatively and quantitatively. The presence of allelochemicals negatively affects the neighboring or successional plants. There is a need to present information to farmers concerning *C. obliqua* and their allelopathic sound effects

*Corresponding author. E-mail: k.musharaf@gmail.com.

Table 1. Allelopathetic effects of *Chrozophra Obliqua* on germination of Bajra.

Extract	Duration	24 h		48 h		Means			
	Concentration	5 g	10 g	5 g	10 g	M1	M2	M3	M4
Control		100	100	100	100	100	100	100	100
Leave		82	70	80	22+	63.5*	76*	51+	46+
Stem		88	88	66	78	80	88	72	66.5
M5		88		74*					

M1, Mean of each extract (5 + 10 g); M2, mean of 5 and 10 g in 24 h; M3, mean of (5 + 10 g) leaves extract in 48 h; M4, mean of 10 g (24 and 48 h); M5, mean of 24 h (5 + 10 g) and 48 h (5 + 10 g) (extract).

Table 2. Analysis of variance of germination of Bajra.

Source	Degrees of freedom (df)	Sum of squares	Mean square	F value	Probability
Replication	4	743.333	185.833	1.5208	0.2127
Factor A	2	13363.333	6681.667	54.6795	000
Factor B	1	2801.667	2801.667	22.9275	000
AB	2	1603.333	801.667	6.5604	0.0032
Factor C	1	1401.667	1401.667	11.4706	0.0015
AC	2	4903.333	2451.667	20.0632	000
BC	1	481.667	481.667	3.9417	0.0534
ABC	2	2343.333	1171.667	9.5883	0.0003
Error	44	5376.667	122.197		
Total	59	33018.333			

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

MATERIALS AND METHODS

Collection of plant materials

C. obliqua was collected from District Karak, Pakistan. Plants were washed thoroughly with water and dried in open air and under natural condition. Leaf and stem samples were separately powdered and stored in plastic bottles at room temperature for use in following experiments.

Experimental protocol

Five and 10 g powdered leave and stems soaked in 100 ml distilled water for 24 h at the room temperature. Aqueous extract were filtrated and final volume was adjusted to 100 ml. The extract was considered as stock solution. Ten healthy and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *Pennisetum typhoides* were kept for germination in sterilized petri-dishes on 2-folds of paper and moistened with 10 ml extracts. Each treatment had 5 replicates. Control consisted of distilled water. The petri-dishes were maintained under laboratory conditions at 25°C temperature with diffused light during day. Equal volume of distilled water was added in the dishes when moisture content of the blotting paper declined. After 72 h, germination and numbers of seminal roots were counted and length of roots and shoots were measured (cm).

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 = 54.6795), 48 h treatment (F = 22.9275) and 10 g concentration (F = 11.4706) on germination. Comparison of extract (Stem and leave of *C. obliqua*) and duration (F = 6.5604) and extracts and concentration (F = 20.0632) showed significant effect of 48 h treatment and 10 g concentration of extract respectively. Comparison of extracts, duration and concentration showed significant effects of 10 g concentration of leaves in 48 h treatment (F = 9.5883), while the comparison of concentration and duration was found insignificant (F = 3.9417) (Tables 1 and 2).

Effect on plumule growth

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves (F = 37.3644), 48 h treatment (F =52.1974)

Table 3. Allelopathetic effects of *Chrozophra obliqua* on plumule length of Bajra.

Extract	Duration	24 h		48 h		Means			
	Concentration	5 g	10 g	5 g	10 g	M1	M2	M3	M4
Control		51.24	51.24	51.24	51.24	51.24	51.2	51.2	
Leave		45.02	29.20	24.02	5.60	26.21*	14.8^{+}	17.3^{+}	36.67*
Stem		61.12	55	29.20	27.80	43.28	28.5	41.4	
M5		48.97		31.52*					

M1, Mean of each extract of 5 + 10 g; M2, mean of 5 and 10 g in 48 h; M3, mean of 10 g of 24 and 48 h; M4, mean of 5 g in 24 and 48 h (leaves extract), M5, mean of 24 h (5 + 10 g) and 48 h (5 + 10 g) (extract).

Table 4. Analysis of variance of plumule length of Bajra.

Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
Replication	4	297.521	74.38	0.8497	
Factor A	2	6541.649	3270.825	37.3644	000
Factor B	1	4569.283	4569.283	52.1974	000
AB	2	2398.885	1199.443	13.7019	000
Factor C	1	761.841	761.841	8.7029	0.0051
AC	2	861.169	430.585	4.9188	0.0118
BC	1	4.056	4.056	0.0463	
ABC	2	26.992	13.496	0.1542	
Error	44	3851.691	87.538		
Total	59	19313.087			

Table 5. Allelopathetic effects of *Chrozophra obliqua* on radical length of Bajra.

	Duration	2 4 h		48 h		Means		
Extract	Concentration	5 g	10 g	5 g	10 g	M1	M2	M3
Control		80.08	80.08	80.08	80.08	80.08	80.08	38.35*
Leave		29.03	7.58	5.26	2.00	10.97*	3.63^{+}	
Stem		74.82	53.84	21.34	6.52	39.13	13.91	
M4		54.23		32.55*				

M1, Mean of each extract of 5 + 10 g; M2, mean of 5 and 10 g in 48 h; M3, mean of 10 g extract in 24 and 48 h; M4, mean of 5 and 10 g in 24 and 48 h each.

and 5 g concentration (F = 8.7029) on plumule length. Comparison of extract and duration (F = 13.7019) and extracts and concentration (F = 4.9188) showed significant effect of 48 h treatment and 10 g concentration of extract respectively. Comparison of extracts, duration and concentration (F = 0.1542) and concentration and duration (F = 0.463) were non significant (Tables 3 and 4).

Effect on radical growth

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves (F =132.0697), 48 h treatment (F = 38.5801) and 10 g concentration (F = 8.3419) on radical length. Comparison of extract and duration (F = 18.3672) showed significant effect of 48 h treatment. While the comparison of extracts and concentration (F = 2.2956), extracts, duration and concentration (F = 9.5883) and concentration and duration (F = 3.9417) were found non significant (Tables 5 and 6).

Effect on number of seminal roots

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves (F = 611.0425), 48 h treatment (F = 46.2068) and 10 g concentration (F = 35.1623) on number of seminal roots. Comparison of extract and duration (F = 18.4702) and extracts and concentration (F

Table 6. Analysis of variance of radical length of Bajra.

Source	Degrees of freedom	Sum of squares	Mean square	F value	Probability
Replication	4	446.309	111.577	0.6101	
Factor A	2	48307.341	24153.67	132.0697	000
Factor B	1	7055.757	7055.757	38.5801	000
AB	2	6718.21	3359.105	18.3672	000
Factor C	1	1525.608	1525.608	8.3419	0.006
AC	2	839.672	419.836	2.2956	0.1126
BC	1	247.051	247.051	1.3508	0.2514
ABC	2	213.976	106.988	0.585	
Error	44	8046.972	182.886		
Total	59	73400.897			

Table 7. Allelopathetic effects of *Chrozophra obliqua* on number of seminal root measure of Bajra.

Extract	Duration	24 h		48 h		Means			
	Concentration	5 g	10 g	5 g	10 g	M1	M2	M3	M4
Control		2.52	2.52	2.52	2.52	2.52	1.27*	2.52	
Leave		0.84	0.70	0.80	0.22^{+}	0.64*		0.46^{++}	0.51^{+}
Stem		1.92	0.88	0.66	0.78	1.06		0.83	
		1.76	1.37	1.33	1.17				
M5		1.56		1.25*					

M1, Mean of each extract of 5 + 10 g; M2, mean of 10 g in 24 and 48 h; M3, mean of 10 g extract in 24 and 48 h (leaves extract); M4, mean of 5 + 10 g in 48 h (leaves extract); M5, mean of 5 and 10 g in 24 and 48 h each.

Table 8. Analysis of variance of number of seminal roots of Bajra.

Source	Degrees of freedom	Sum of squares	Mean square	F Value	Probability
Replication	4	0.166	0.041	1.2995	0.285
Factor A	2	38.949	19.475	611.0425	000
Factor B	1	1.473	1.473	46.2068	000
AB	2	1.177	0.589	18.4702	000
Factor C	1	1.121	1.121	35.1623	000
AC	2	0.585	0.293	9.1828	0.0005
BC	1	0.216	0.216	6.7773	0.0125
ABC	2	1.708	0.854	26.7953	000
Error	44	1.402	0.032		
Total	59	46.797			

= 9.1828) showed significant effect of 48 h treatment and 10 g concentration of extract respectively. Comparison of extracts, duration and concentration showed significant effects 10 g concentration of leaves in 48 h treatment (F= 26.7953). The comparison of concentration and duration was also found significant (F = 6.7773) (Tables 7 and 8).

DISCUSSION

In the present study allelopathic sound effects of *C. obliqua* was observed on germination and seedling growth of Bajra. From preface screening it was initiate that leaf and stem extract had the strongest allelopathic effect on seed germination. Xuan et al., (2004) also found that the inhibitory allelopathic impact of leaves extract was more powerful than of other vegetative parts. The study demonstrated that leaves aqueous extracts of *C. obliqua* exhibited significant inhibitory effects on seed germination and seedling growth of Bajra. This indicates the accessibility of the inhibitory chemicals in higher concentration in leaves than in stem.

The comparative analysis between soaking duration and extract concentration showed that 48 h treatment of 10 g leaves extract have produced more inhibitory effect on germination, radical and plumule growth and number of seminal roots of Bajra. Its effectiveness on germination and growth suggests that leaves and stem of *C. obliqua* 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.

It has been seen that many species release phytotoxic substances before decay but generally plant litter increases soil fertility during 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 Bajra. 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 physiologicaly activity level (Hussain et al., 2004). 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 *C. obliqua* in experiments significantly inhibited Bajra. These findings have the same opinion with those of Hussain et al. (2004) and Khan et al. (2011) who also observed comparable phytotoxicity by other plants.

The observed different phytotoxicity of C. oblique may be attributed to the presence of variable amount of phototoxic substances in different parts that leach out under natural conditions. Some recent studies indicating the phytotoxic/ allelopathic effect of aqueous extracts of weeds include *Mikania micrantha* (Ismail and Kumar, 1996), *Vulpia* spp. (An et al., 1999), *Cyperus rotundus* (Quayyum et al., 2000), *Cardaria draba* (Kiemnec and McInnis, 2002), *Parthenium hysterophorus* (Singh et al., 2003), *Brassica nigra* (Tawaha and Turk, 2003), *Raphanus raphanistrum* (Norsworthy, 2003), *Ageratum conyzoides* (Batish et al., 2002), *Rhazya stricta* (khan et al., 2011) and *C. obliqua* (Khan et al., 2011). All these studies point out the release of phototoxic chemicals at some stage in the preparation of aqueous extracts. Based on this, studies were further extended to search the impact of *C. obliqua* leaves, as they possessed better phytotoxicity on the appearance and growth of weed plants.

Conclusions

The present investigation exposed that its effectiveness on germination and seedling growth suggests that leaves of *C. obliqua* may operate as a source of allelochemicals after being released into soil or after decomposition. The presence of allelochemicals negatively affects the neighboring or successional plants. There is a need to present information to farmers concerning *C. obliqua* and their allelopathic sound effects. Further studies are suggested to spell out the prospective physiological mechanism allied to allelopathic achieve on plants.

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