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Effect of aqueous extracts of *Chrozophora obliqua* (Del.) Juss. on germination and seedling growth of *Zea mays*

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Abstract

The allelopathic influence of aqueous extracts of *Chrozophora obliqua* (Del.) Juss. on the germination and seedling growth of *Zea mays* have been determined. It was noted that 10g aqueous extracts of leaves and 48hr treatment present significant inhibitory effect on germination, radical length and number of seminal roots and the effect 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 5g concentration of leaves that decreased the plumule length. These findings indicate that *Z. mays* 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.

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Introduction

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 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). 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). 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 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 *Z. mays* 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 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 the yield both qualitatively and quantitatively.

Materials and methods

Collection of plant materials

Chrozophora 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

From preliminary screening it was found that leaf and stem extracts have the strongest allelopathic effect on seed germination; thus we selected leaves and stem for more accurate experiments. Five and 10g powdered leave and stems soaked in 100ml distilled water for 24h at the room temperature. Aqueous extract were filtrated and final volume was adjusted to 100 ml. The extract was considered as stock solution. 10 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 paper and moistened with 10ml 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 7 days, germination was counted and length of roots and shoots were measured (cm) and numbers of seminal roots 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 inhibitory effects of leaves ($F=4.1320$, $P< 0.05$) and 24h treatment ($F=5.4721$, $P<0.05$). Comparison of extract and duration, extracts and

concentration, Comparison of extracts, duration and concentration and concentration and duration were insignificant. (Table 1a and 1b).

Table 1a. Allelopathetic effects of *Chrozophra obliqua* on germination of Maize.

Extract	Duration	24hr		48hr		Means
	Conc.	5g	10g	5g	10g	M1
	Control	100	100	100	100	100
	Leave	96	92	98	100	96.5*
	Stem	94	100	98	100	98
		96.6	97.3	98.6	100	
		97*		99.3		

*: within group + : between group

Table 1b. Analysis of variance of *Chrozophra obliqua* on germination of Maize.

Source	Deg. of Freedom	Sum of Sqr.	Mean Sqr.	F Value	Probability
Reps.	4	23.33	5.83	0.39	
Factor A	2	123.33	61.66	4.13	0.02
Factor B	1	81.66	81.66	5.47	0.02
AB	2	63.33	31.66	2.12	0.13
Factor C	1	15	15	1.00	0.32
AC	2	70	35	2.34	0.10
BC	1	1.66	1.66	0.11	
ABC	2	63.33	31.66	2.12	0.13
Error	44	656.66	14.92		
Total	59	1098.33			

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=64.8822, P< 0.05), 48h treatment (F= 4.9130, P< 0.05) and 10g concentration (F= 6.8488, P< 0.05) on plumule growth. Comparison of extracts and concentration (F= 4.0375, P< 0.05) showed significant differences of 10g concentration of leave extract. (Table 2a and 2b).

Effect on radical growth

Three way ANOVA (RCBD) (df 1, 44) showed significant effects of leaves (F=131.9299, P< 0.05), 48h treatment (F= 72.204, P< 0.05) and 10g concentration (F=47.8793, P<0.05) on radical growth. Comparison of extract and duration (F=18.1548, P< 0.05) and extracts and concentration (F= 16.151, P< 0.05) showed significant differences of 48h treatment and 10g concentration of extract respectively (Table 3a and 3b).

Table 2a. Allelopathetic effects of *Chrozophra obliqua* on plumule length of Maize.

Extract	Duration	24hr		48hr		Means		
	Conc.	5g	10g	5g	10g	M1	M2	M3
	Control	69.0	69.0	69.0	69.0	69.0		
	Leave	43.3	32.5	44.7	23.8	36.0*		
	Stem	55.8	50.1	40.8	40.3	46.7	47.4*	28.1+
		56.0	50.5	51.5	44.4			
		53.32		47.97*				

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

Table 2b. Analysis of variance of *Chrozophra obliqua* on plumule Length of Maize.

Source	Deg. of Freedom	Sum of Sqr.	Mean Sqr.	F Value	Probability
Reps.	4	504.3	126.0	1.4	0.2
Factor A	2	11339.9	5669.9	64.8	0
Factor B	1	429.3	429.3	4.9	0.03
AB	2	406.0	203.0	2.3	0.1
Factor C	1	598.5	598.5	6.8	0.01
AC	2	705.6	352.8	4.0	0.02
BC	1	10.3	10.3	0.1	
ABC	2	149.9	74.9	0.8	
Error	44	3845.0	87.3		
Total	59	17989.1			

Table 3a. Allelopathetic effects of *Chrozophra obliqua* on Radical growth of Maize.

	Duration	24hr		48hr		Means			
Extract	Conc.	5g	10g	5g	10g	M1	M2	M3	M4
	Control	72.9	72.9	72.9	72.9	72.9			
	Leave	63.3	27.2	28.5	6.3	31.3*	44.6*	17.4+	16.8+
	Stem	71.8	56.4	45.2	32.0	51.4			
		69.3	52.2	48.9	37.1				
	M5	60.80		43.03*					

Table 3b. Analysis of variance of *Chrozophra obliqua* on radical length of Maize.

Source	Deg. of Freedom	Sum of Sqr.	Mean Sqr.	F Value	Probability
Reps.	4	358.9	89.7	1.3	0.2
Factor A	2	17299.5	8649.7	131.9	0
Factor B	1	4733.9	4733.9	72.2	0
AB	2	2380.5	1190.2	18.1	0
Factor C	1	3139.1	3139.1	47.8	0
AC	2	2117.8	1058.9	16.1	0
BC	1	106.5	106.5	1.6	0.2
ABC	2	139.5	69.7	1.0	0.3
Error	44	2884.7	65.5		
Total	59	33160.8			

Table 4a. Allelopathetic effects of *Chrozophra obliqua* on number of seminal roots of Maize.

Extract	Duration	24hr		48hr		Means	
	Conc.	5g	10g	5g	10g	M1	M2
	Control	6.16	6.1	6.1	6.1	6.1	
	Leave	4.5	4.4	5.4	2.26+	4.1	3.3*
	Stem	4.8	4.8	5.4	5.52	5.1	
		5.1	5.1	5.6	4.6+		
	M5	5.15		5.17			

Table 4b. Analysis of variance of *Chrozophra obliqua* on number of seminal roots of Maize.

Source	Deg. of Freedom	Sum of Sqr.	Mean Sqr.	F Value	Probability
Replication	4	0.9	0.2	0.6	
Factor A	2	39.8	19.9	55.3	0
Factor B	1	0.0	0.0	0.0	
AB	2	3.9	1.9	5.4	0.007
Factor C	1	4.3	4.3	12.0	0.001
AC	2	9.6	4.8	13.4	0
BC	1	3.9	3.9	10.8	0.002
ABC	2	8.1	4.0	11.2	0.0001
Error	44	15.8	0.3		
Total	59	86.4			

Effect on number of seminal roots

Three way ANOVA (RCBD) (df 1, 44) showed significant inhibitory effect of 10g concentration (F= 12.0197, P< 0.05) on number of seminal roots. Comparison of extract and duration (F=5.4397, P< 0.05) and duration and concentration (F= 10.8549, P< 0.05) showed significant differences of 48h treatment and 10g concentration of extract respectively (Table 4a and 4b).

Discussion

In the present study allelopathic effects of *C. obliqua* was observed on germination and seedling growth of *Zea mays*. From preliminary screening it was found that leaf and stem extract had the strongest allelopathic effect on seed germination. Tefera (2002) 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 *Zea mays*. This indicates the availability of the inhibitory chemicals in higher concentration in leaves than in stem.

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 & Bansal (2002), who reported that *Ageratina adenphora* litter reduced growth of *Lantana camara*. Similarly, Maciel *et al.*, (2003) also reached to similar results.

The comparative analysis between extract, concentration and soaking duration showed that 48h treatment of 10g leaves extract have produced more inhibitory effect on germination, plumule and radical growth and numbers of seminal roots of *Zea mays*. Similarly, the phytotoxicity *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), generally enhanced with soaking duration, and this supports our findings. 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. 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.

Allelopathic substances released by the plants accumulate in the soil to physiologically activity level (Hussain *et al*., 2004) 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 *Chrozophora obliqua* in experiments significantly inhibited test species. These findings agree with those of Hussain *et al*., (2004) and Eppard *et al*., (2005) who also observed similar phytotoxicity by other plants.

Some recent studies indicating the phytotoxic/ allelopathic effect of aqueous extracts of weeds include *Mikania micrantha* (Ismail and Kumar, 1996), *Vulpia sp*. (An et al., 1999), *Cyperus rotundus* (Quayyaum et al., a2000), *Cardaria draba* (Kiemnec and McInnis, 2002), *Parthenium hysterophorus* (Singh et al., 2003a), *Brassica nigra* (Tawaha and Turk, 2003), *Raphanus raphanistrum* (Norsworthy, 2003) and *Ageratum conyzoides* (Batish et al b., 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 *C. obliqua* (especially) leaves, as they possessed greater phytotoxicity on the emergence and growth of weed plants.

Conclusion

The present investigation revealed that aqueous extract of *C. obliqua* at different concentration levels inhibited the germination percentage, radical length, Plumule length and number of seminal roots of *Zea mays* seedlings. Its effectiveness on germination and growth suggests that leaves of *C. obliqua* may act as a source of allelochemicals after being released into soil or after decomposition. Further studies are suggested to clarify the possible physiological mechanism related to allelopathic effect on plants.

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