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Allelopathic potential of *Diospyros kaki* L. against *Triticum aestivum* L., *Brassica compestris* L. and *Trifolium alexandrinum* L.

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Abstract

Allelopathic studies of *Diospyros kaki* L. were conducted by using aqueous extracts from leaves, bark, litter and mulching in various experiments. It was observed that the germination, radical growth, plumule growth, no. and growth of seminal roots, fresh weight and dry weight of *Triticum aestivum* L and *Brassica campestris* L. and *Trifolium alexandrinum* L. were significantly reduced in various bioassays. The aqueous extracts of leaves were inhibitorier than the bark extracts for the test species *Triticum aestivum* L. Aqueous extracts obtained at room temperature were inhibitorier than hot water extracts. Litter and mulching experiments also showed inhibitory effects. Further studies are required to see its allelopathic behavior under field condition against its associated species, to identify the toxic principle, and to evalvate as biocontrol agents for weeds, insect and disease control.

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Introduction

Allelopaty is direct or indirect effect of one plant to adjoining plants through release of chemical substances (Rice 1979). Allelopathy is "the release of phytotoxins by plants" (Bais *et al.*, 2003). Allelopathy affects plant distribution, community formation, intercrop evolution and biodiversity conservation (Peneva 2007). Allelochemical can be present in root, stem, leaves, flowers and fruits, and inhibit root growth, shoot growth, germination percentage, nutrients uptake (Murawat and khan 2006).

Many plant species such as *Terminalia bellirica, Terminalia chebula, Aegle marmelos* and *Sapindus mukorossi* (Thapaliyal *et al.,* 2008); *Ficus subincisa* , *Bauhinia purpurea*, and *Toona hexandra (*Sing *et al., 2009); Cenchrus ciliaris* and *Bothriochloa pertusa* (Hussain *et al.,* 2010); *Dodonaea viscose (* Barkatullah *et al.,* 2010); *Juniperus ashei* (Young & bush 2009); *Azadirachta indica* (Ashrafi *et al.,* 2008); *Cassia angustifolia (*Hussain *et al.,* 2007*)and Cenchrus ciliaris and Bothriochloa pertusa (*Hussain & Ilahi 2009) allelopathic effect on test species. The other workers, (Khan *et al.,* 2005; Hussain *et al.,* 2007; Maharjan *et al.,* 2007; Uddin *et al.,* 2007, Salih *et al.,* 2009; Sun *et al* 2006; Kumar et al., 2006; Terzi 2008; khan *et al.,* 2005; Thapaliyal *et al.,* 2008). Also conducted Allelopathic studies of literature reveals that no such study was conducted on *Diospyros kaki* L.

Diospyros kaki Linn (Family Ebenaceae) is Small cultivated tree, up to 15 m tall, originating from Japan, Introduced and cultivated throughout Eastern Asia, Russia, Japan and China. The original habitat of the species is China, but it has been introduced and cultivated elsewhere. It also cultivated in Pakistan for its edible fruit, especially in Swat, Dir and Malakand. The aim of the study was to check out the Allelopathic potential of *Diospyros kaki* Linn against some selected valuable crops.

Material and method

Mature leaves and bark of *Diospyros kaki* L. were collected from Swat, dried at room temperature (25˚C- 30˚C) and powdered, Glass wares were washed with tap water and sterilized at 170˚C for at least 4 hours. All the results were statistically analyzed through one way ANOVA.

Effect of aqueous extracts

Five and 10 gm of each part were separately soaked in 100 ml distilled water at 25˚C for 24 and 48 hours respectively and filtered to get aqueous extracts. These extracts were used against *Triticum aestivum* L., *Brassica campestris* L. and *Trifolium alexandrinum* L. used as the test species on 2-fold filter paper in petri dishes following standard filter paper bioassay (Hussain & Ilahi 2009; Barkatullah *et al.,* 2010, Hussain *et al.,* 2010). The filter papers were moistened with the aqueous extracts, while distilled water was used as a control. For each treatment, five replicates, each with 10 seeds were made. The petri dishes were incubated at 25˚C. After 72 hours, the percent germination, length of plumule and radical was noted. Twenty seedlings were randomly taken for fresh and dry weight determination and moisture contents. Seedlings were dried at 65˚C for 72 hours for the determination of dry weight and moisture contents.

Effect of hot water extracts

Five gm and 10gm of dried plant parts were separately boiled in 100 ml water for 5 minutes and filtered. The room cooled extracts were applied against the same test species as before.

Effect of litter

Five gm of litter each from leaves and bark were crushed and placed over one fold of filter paper in a petri dish. The filter papers were moistened with 5 ml water. In control treatment fine pieces of filter paper were used instead of plant material.

Table 1. Effect of cold aqueous extract on germination, plumule and radical growth of test species. Each value is a mean of 5 replicates each with 10 seedlings.

Test species	*Triticu aestivum* L.				*Brassica compestris* L.				*Trifolium alexandrinum* L.			
Soaking duration and concentration	5g/ 24h	5g/ 48h	10g/24h	10g/48h	5g/ 24h	5g/ 48h	10g/24h	10g/48h	5g/ 24h	5g/ 48h	10g/24h	10g/48h
					Germination %							
Control	82	82	82	82	92	92	92	92	94	94	94	94
Bark	78	84	68*	80	82	72	88	86	100	96	90	100
% of control	95.12	102.43	82.92	97.56	89.13	78.26	95.65	93.47	106.38	102.12	95.74	106.38
Leaves	74	70	94	88	58***	40***	22***	14***	92	98	96	94
% of control	90.24	85.36	114.63	107.31	63.04	43.47	23.91	15.21	97.87	104.25	10212	100
					Plumule growth (mm)							
Control	8.18	8.18	8.18	8.18	6.9	6.9	6.9	6.9	5.68	5.68	5.68	5.68
Bark	5.76**	3.78***	5.56***	3.7***	4.26***	1.18***	2.82***	1.54***	8.26	4.56	6	5.76
% of control	70.14	46.21	67.97	45.23	61.73	17.10	40.86	23.04	145.42	80.28	105.63	101.40
Leaves	3.78***	1.86***	3.8***	1.62***	1.54***	0.58***	0.32***	0.20***	2.22***	2.8***	1.76***	1.96***
% of control	46.21	22.73	46.45	19.80	22.31	8.40	3.91	2.89	39.08	49.29	30.98	34.50
					Radical growth (mm)							
Control	14.84	14.84	14.84	14.84	9.52	9.52	9.52	9.52	8.16	8.16	8.16	8.16
Bark	12.94	7.74***	13.7	7.82***	5.96***	2.98***	5.02***	4.16***	7.6	7.18	5.72***	9.34
% of control	87.19	52.15	92.31	52.69	62.60	31.30	52.73	43.69	93.13	87.99	70.09	114.46
Leaves	12.62	7.94***	14.54	6.92***	4.04***	1.06***	0.92***	0.36***	3.94***	6.12***	4.0***	6.22***
% of control	85.04	53.50	97.97	46.63	42.43	11.13	9.6	3.78	48.28	75.0	49.01	76.22
					Seminal root growth (mm)							
Control	11.56	11.56	11.56	11.56	0	0	0	0	0	0	0	0
Bark	8.65*	3.94***	8.64*	4.14***	0	0	0	0	0	0	0	0
% of control	74.82	34.08	74.74	35.81	0	0	0	0	0	0	0	0
Leaves	8.86*	2.32***	9.16*	2.92***	0	0	0	0	0	0	0	0
% of control	76.64	20.06	79.23	25.25	0	0	0	0	0	0	0	0
					No of Seminal root							
Control	1.52	1.52	1.52	1.52	0	0	0	0	0	0	0	0
Bark	1.46	1.64	1.32	1.30	0	0	0	0	0	0	0	0
% of control	96.05	107.89	86.84	85.52	0	0	0	0	0	0	0	0
Leaves	1.38	1.46	1.92	1.72	0	0	0	0	0	0	0	0
% of control	90.78	96.05	126.31	113.15	0	0	0	0	0	0	0	0

Effect of mulching

Five gm each of the crushed dried leaves and bark were placed in plastic cups which were half filled with sterilized moist sand. For each treatment five replicate, each with 10 seeds were made. Control consisted of fine pieces of filter paper. The plastic cups were incubated at 25˚C and observed for germination. After 7 days growth of plumule and radical were measured. Twenty seedlings were randomly taken for fresh and dry weight and moisture contents.

Results and discussion

Effect of aqueous extracts

Percent germination was not affected by the application of aqueous extracts of both bark and leaves in all test species (Siddiqui *et al.,* 2009), but radical and plumule length, fresh weight, dry weight and moisture contents are declined (Table 2). (Barkatullah *et al.,* 2010; Hussain et al 2010: Samreen *et al.,* 2009, hussain & ilahi 2009) have also reported similar results for allelopathic effects of various plants. Except 5g/24h aqueous extract percent germination in *Triticum aestivum* L. and the aqueous extracts of leaves reduce germination in *Brassica compestris* L. (Alagesaboopathi. 2010; Uniyal & Chhetri 2010). Plumule, radical and percent germination are not effected by application of aqueous extracts of bark in *Trifolium alexandrinum* L. Seminal roots were also inhibited in *Triticum aestivum* L. More inhibition occured by increasing concentration of the aqueous extracts. (Samreen *et al.,* 2009; Hussain *et al.,* 2010 and Barkatullah *et al.,* 2010).

Table 2. Effect of aqueous extract on fresh weight, dry weight and moisture content of seedlings. Each value is a mean of 20 randomly selected seedlings.

Fresh weight (mg)												
Control	2322	2322	2322	2322	408.1	408.1	408.1	408.1	319.1	319.1	319.1	319.1
Bark	2003.6	1587.2	2122	1592	175.6	128.3	211.4	161.4	299.6	193.9	299.8	185.4
% of control	86.28	68.35	91.38	68.56	43.02	31.43	51.80	39.54	93.88	60.76	93.95	58.10
Leaves	1798.3	1364.8	1869.4	1498.8	156.3	0	0	0	205.5	184.1	211.8	136
% of control	77.44	58.77	80.50	64.54	38.29	0	0	0	64.39	57.69	66.37	42.61
Dry weight (mg)												
Control	1249.6	1249.6	1249.6	1249.6	42.1	42.1	42.1	42.1	31.5	31.5	31.5	31.5
Bark	1058.9	935.8	1152.4	974.4	18.6	23.1	31.2	29.2	22.4	29.1	37.1	23.6
% of control	84.73	74.88	92.22	77.97	44.18	54.86	74.10	69.35	71.11	92.38	117.7	74.92
Leaves	1109.2	905.2	1155.3	932.9	31.8	0	0	0	25.1	28.1	34.6	27.8
% of control	88.76	72.43	92.45	74.65	75.53	0	0	0	79.68	89.20	109.84	88.25
Moisture contents (mg)												
Control	85.86	85.86	85.86	85.86	869.35	869.35	869.35	869.35	913.01	913.01	913.01	913.01
Bark	89.21	69.60	84.08	63.38	844.08	455.4	577.56	455.73	1237.5	566.32	708.08	685.59
% of control	96.24	81.06	97.92	73.81	97.09	52.38	66.43	52.42	135.54	62.02	77.55	75.09
Leaves	62.12	50.77	100.3	60.66	391.5	0	0	0	718.72	555.16	512.13	389.2
% of control	72.35	59.13	116.81	70.64	45.03	0	0	0	78.71	60.80	56.09	42.62

*Significantly different from control at alpha 0.050 according to one way ANOVA
(*less significant, **moderately significant, ***highly significant)

The inhibition of early growth of seedling is a critical step by the aqueous extracts. In many cases germination may be stimulated but seedling growth is strongly reduced. In present case the plumule and radical growth of both test species was significantly retarded in all the test conditions (Table 1). These findings agree with those of Khan *et al.,* (2008); Barkatullah *et al*., (2010); Hussain *et al.,* (2010), who observed inhibited growth of seedlings by inhibitors from other plants.The present results are also in line with those of Uniyal & Sachin (2010), Abugre & Sam (2010) and Hussain & Ilahi (2009) who also observed allelopathic inhibition of radical and plumule growth of test species.

A weak seedling is disadvantage to a growing plant as it cannot properly take up water and minerals from the habitat. The reduction in growth could be due to water loss that leads to poor biomass. It has been seen that inhibited seedlings also have poor fresh and dry weight (Table 2), which means poor accumulation of food and growth. Similar reduction in fresh and dry weight of seedlings has been reported by many workers (Uniyal & Sachin 2010; Barkatullah *et al*., 2010; Hussain *et al.,* 2010: Samreen *et al.,* 2009, hussain & ilahi 2009) and our findings agree with them. The reduction of moisture contents of seedlings mean failure of seedlings to absorb sufficient soil moisture. A drought like condition prevailed imparity in the functioning of root, radical mean poor growth performance as was evident in the present study. Such a reduction in moisture contents as been reported by earlier workers (Hussain *et al* 2010; Barkatullah *et al.,* 2010; Hussain & Ilahi 2009; Uniyal & Sachin 2010; Abugre & Sam 2010).

Table 3. Effect of hot water extract on the germination, plumule and radical growth of test species. Each value is a mean of 5 replicates, each with 10 seedlings.

Test species	*Triticu aestivum L.*		*Brassica compestris L.*		*Trifolium alexandrinum* L.	
Hot water extract and concentration	5g	10g	5g	10g	5g	10g
			Germination %			
Control	82	82	92	92	94	94
Bark	84	80	92	94	100	98
% of control	102.43	97.56	100	102.17	106.38	104.25
Leaves	74	84	64	68	96	98
% of control	90.24	102.43	69.56	73.91	102.12	104.25
			Plumule growth (mm)			
Control	8.18	8.18	6.9	6.9	5.68	5.68
Bark	5.84	7.28	2.62***	2.7***	1.3***	16.16***
% of control	71.39	88.99	37.97	39.13	22.88	284.50
Leaves	2.76***	3.26***	0.82***	0.92***	4.64	3.26***
% of control	33.74	39.85	11.88	13.33	81.69	57.39
			Radical growth (mm)			
Control	14.84	14.84	9.52	9.52	8.16	8.16
Bark	16.36	16.42	7.76	10.04	16.3	14.06
% of control	110.24	110.64	81.51	105.46	199.75	172.30
Leaves	9.28*	8.9*	2.62***	2.42***	3.28***	2.72***
% of control	62.53	59.97	27.52	25.42	40.19	33.33
			Seminal root growth (mm)			
Control	11.56	11.56	0	0	0	0
Bark	11.26	10.98	0	0	0	0
% of control	97.40	94.98	0	0	0	0
Leaves	6.38*	6.94	0	0	0	0
% of control	55.19	60.03	0	0	0	0
			No of Seminal root			
Control	1.52	1.52	0	0	0	0
Bark	1.64	1.48	0	0	0	0
% of control	107.89	97.36	0	0	0	0
Leaves	1.26	1.42	0	0	0	0
% of control	82.89	93.42	0	0	0	0

Effect of hot water extracts

Use of hot water extracts is unusual in natural environment, but to facilitate and save time, similar studies have been made (Hussain *et al.,* 2010; Barkatullah *et al*., 2010; Samreen *et al.,* 2009; Hussain & Ilahi 2009). In the present study hot water extract significantly caused inhibition (Table 3), Seminal root were also inhibited in wheat. But the hot water extracts of bark has not affected test species *Triticum aestivum* L. while inhibited plumule growth in *Brassica* and *Trifolium*. The leaf extract inhibited plumule, radical and seminal root in *Triticum aestivum* L., plumule, radical and percent germination in *Brassica*. Only 5g hot water leaves extracts inhibited radical growth in *Trifolium alexandrinum* L.

These results intimated that phytotoxins were easily extracted within short time and the phytotoxins retained phytotoxicity after boiling. The present study also showed that hot water extracts effectively exhibited allelopathy. Inspite of all favours, it is an unusual process that hardly can be possible in nature. The results of cold and hot water extracts almost agree with each other and strengthen the view that extracts of *Diospyrus kaki* L. are inhibitory to the test species.

Table 4. Fresh weight, dry weight and moisture content of test species in hot water extract bioassay.

Fresh weight (mg)						
Control	2322	2322	408.1	408.1	319.1	319.1
Bark	1904	1763.6	115.6	99.4	95.3	100.6
% of control	81.99	75.95	28.32	24.35	29.86	31.52
Leaves	1581.2	1616	225	61.3	124.6	39.6
% of control	68.09	69.59	55.13	15.02	39.04	12.40
Dry weight (mg)						
Control	1249.6	1249.6	42.1	42.1	31.5	31.5
Bark	1015.3	943	37.8	40.2	25.7	21.3
% of control	81.25	75.46	89.78	95.05	81.58	67.61
Leaves	1009.8	992.3	39.9	44.1	26.9	26.4
% of control	80.80	79.40	94.77	104.75	85.39	83.80
Moisture contents (mg)						
Control	85.86	85.86	869.35	869.35	913.01	
Bark	87.56	87.02	205.8	147.2	326.76	
% of control	101.97	101.35	23.67	16.93	35.78	
Leaves	56.58	62.85	463.9	39.0	363.19	
% of control	65.89	73.20	53.36	4.48	39.77	

*Significantly different from control at alpha 0.050 according to one way ANOVA
(*less significant, **moderately significant, ***highly significant)

Table 5. Effect of litter and mulching on germination, plumule and radical growth of test seedlings. Each value is a mean of five replicates, each with 10 seedlings.

Treatment	Litter			Mulching		
Test species	*Triticum aestivum L.*	*Brassica compestris l.*	*Trifolium alexandrinum* L.	*Triticum aestivum L.*	*Brassica compestris L.*	*Trifolium alexandrinum* L.
			Germination %			
Control	82	92	94	76	68	84
Test	86	76	96	42	40**	80
% of control	104.87	82.60	102.12	55.26	58.82	95.23
			Plumule growth (mm)			
Control	8.18	6.9	5.68	74.98	7.7	22.42
Test	5.52	3.86***	5.46	10.9***	3.38***	21.28
% of control	67.48	55.94	96.12	14.53	43.89	94.91
			Radical growth (mm)			
Control	14.84	9.52	8.16	31.24	30.56	10.38
Test	15.26	8.46	9.02	5.12***	11.44***	12.5
% of control	102.83	88.86	110.53	16.38	37.43	120.42
			Seminal root growth (mm)			
Control	11.56	0	0	20.48	0	0
Test	10.62	0	0	3.14***	0	0
% of control	91.86	0	0	15.33	0	0
			No. of Seminal root			
Control	1.52	0	0	4.3	0	0
Test	1.68	0	0	1.84	0	0
% of control	110.52	0	0	42.79	0	0

Effect of mulching and litter

It is commonly understood that litter improves soil nutrients and physiochemical features of soil. However, it is also agreed (Inderjit & Duke 2003; Sasikumar *et al.,* 2001; Hussain & Ilahi 2009; Barkatullah *et al.,* 2010; Samreen *et al.,* 2009; Hussain *et al.,* 2010) that litter prior to decay might release phytotoxins in the soil. This possibility was envisaged by performing litter and mulching experiments. In both these experiments it was seen that only plumule growth are inhibited by mulch in *Triticum aestivum* L. *Brassica compestris* L. while there was no effect on *Trifolium alexandrinum* L. (Table 5). Litter inhibited percent germination, plumule and radicle growth of *Triticum aestivum* L. *Brassica compestris* L. while *Trifolium alexandrinum* L. was not affected. The fresh and dry weight and moisture contents of test species, in both the experiments decreased significantly (Table 6).

Table 6. Effect of added litter and mulch on the fresh weight, dry weight and moisture contents of seedlings.

Fresh weight (mg)						
Control	2322	408.1	319.1	2859.9	533	292.9
Test	1904.6	152.3	254.9	1722.6	310	209
% of control	82.02	37.31	79.88	60.23	58.16	71.35
Dry weight (mg)						
Control	1249.6	42.1	31.5	1042	98.9	54.8
Test	1129.8	25.7	25.6	896.3	39.1	54.4
% of control	90.41	61.04	81.26	86.01	39.53	99.27
Moisture content (mg)						
Control	85.86	869.35	913.01	174.46	438.9	434.48
Test	68.57	492.6	418.43	92.19	692.8	284.1
% of control	79.86	56.66	45.82	52.84	157.84	65.38

*Significantly different from control at alpha 0.050 according to one way ANOVA

(*less significant, **moderately significant, ***highly significant)

It was evident that added litter and mulch proved inhibitory just like the aqueous extracts. The litter and mulch reduced germination, seedling growth and physiological aspects of tested plants. It can be visualized that addition of litter from Diospyros kaki L. might intoxicate the soil as observed in the present case.The litter of *Dodonaea viscosa* (Barkatullah *et al.,* 2010), *Cenchrus ciliaris* and *Bothriochloa pertusa* (Hussain *et al*., 2010) have been reported to exhibit similar inhibition and our findings agree with these. The present investigation also indicated that leaves and bark were differentially toxic to the *Triticum*, *Brassica and trifolium*. These test species had their own differential response towards the same extract. Similar differential behavior of *Cenchrus ciliaris* and *Bothriochloa pertusa* (Hussain & Ilahi 2009), *Calotropis procera (Samreen et al., 2009), Eucalyptus camadulensis* (Mohammad & Rajaie, 2009) has been reported, which support the present findings. Furthermore, phytotoxicity is depended upon the concentration, soaking duration and the physiological responses of test species.

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