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Allelopathic Effects of Walnut Plants (*Juglans regia* L.) on Four Crop Species

Hussain, Farrukh, Ihsan Ilahi and Bong-Seop Kil*
(Phytoecology Laboratory, Department of Botany, University of Peshawar, Peshawar, Pakistan and
**Department of Science Education, Wonkwang University, Iri, Korea)*

네종 작물에 대한 *Juglans regia* L.의 알레로패티 효과

Hussain, Farrukh, Ihsan Ilahi, 吉 奉 燮*
(Phytoecology Laboratory, Department of Botany, University of Peshawar, Peshawar, Pakistan,
*원광대학교 사범대학 과학교육과)

ABSTRACT

Walnut (*Juglans regia* L.) is a common cultivated and wild deciduous tree in the temperate regions of Pakistan and elsewhere. A bare or poor understorey of wild or cultivated species is frequently observed beneath and around it. Soil analysis indicated insignificant differences in nutrient status of soils taken from areas with or without walnut influence. Field investigations revealed that the height, shoot, fresh and dry weight of potato, turnip, corn and bean significantly decreased in the walnut-influenced parts of the field compared to their counter-parts in the same field but without walnut influence.

Aqueous extracts from various parts, shoot litter, natural rain leachates and soil collected from beneath walnut significantly reduced the germination, early seedling growth, fresh and dry weight, and moisture contents of corn, turnip and bean in various laboratory experiments. Ferulic, *p*-coumaric, caffeic, vanillic, *p*-hydroxybenzoic, chlorogenic and gallic acid were identified as the possible allelopathic substances in the aquous extracts and rain leachates of the walnut tree. The findings suggest that the observed poor growth of crops is due to allelopathic effects of walnut.

INTRODUCTION

Walnut (*Juglans regia* L.) is a common cultivated and wild deciduous tree in the temperate forests of Pakistan and elsewhere. The tree is interspersed within the main forest types. The plant is usually grown on wastelands and cultivated fields due to its valuable drupes, barks and woods. Leaves, floral parts and fruits are regular source of litter fall every autumn season which ultimately decays in the immediate soil. A bare area and/or a poor unhealthy understorey of wild and crop species is frequently observed beneath and walnut tree. The growth suppressing effects extend to a certain distance beyond the canopy fringes. Various crops including corn, lentil, potato, turnips, beans and *Malva* sp. are generally cultivated in association with walnut trees by the farmers. However, under similar agronomic practices, the plants coming under the influence of walnuts have distinctly low height and poor growth compared to their counterparts in the same field but without the influence of walnut. In Kalam (District Swat) and Kawai (District Mansehra) unhealthy corn, turnip, potato and bean plants were seen under the influence of walnut. This attracted us to explore the possible role of allelopathy by walnut against these associated crop species. Some previous workers like Massey (1925), Davis (1928), Roschina (1937), Ponder (1981), Putnam and Tang (1986), Putnam and Weston (1986) and Fisher (1987) have reported the possibility of allelopathy by walnut species.

Similarly, the creation of bare areas and low productivity of crops and wild species due to allelopathy is well documented phenomena (Lodhi and Rice, 1971; Kil and Yim, 1983; Rice, 1984, Putnam and Tang, 1986; Fisher, 1987).

Keeping in view the observed poor growth of crops in association with walnut and importance of allelopathy in agriculture, the present investigation was, therefore, conducted to find the role of allelopathy by walnut (*Juglans regia* L.) against corn, turnip and bean which are commonly grown with walnuts in the temperate regions especially Kalam-Utror, district Swat, in Pakistan.

MATERIALS AND METHODS

Field Observations. Ten crop fields with walnut trees on it were selected for each of the crops; potato (*Solanum tuberosum* L.), corn (*Zea mays* L.), turnip (*Brassica campestris* subsp. *rapa*) and bean (*Phaselus vulgaris* Linn) in Kalam, District Swat. Twenty plants of every crop species were randomly rooted out from places with or without the influence of walnut in the same field on October 10, 1987. For corn, an additional observation was made on July 7, 1987 to Kalam and on July 15, 1987 to Kawai, district Mansehra. The shoot lenght, fresh and dry mass number of pods, grains/pod, length of pod of bean, ear length, number grains/ear, weight of grains, number and weight of potato tubers, weight of turnips and other parameters listed in Table 2 and 3 were determined for each of the crop species in both the situations.

The top 15 cm soil was collected from Kalam in each of the fields and crops with or without walnut influence. It was air dried, litter removed and analyzed for pH, electrical conductivity, total soluble salts, nitrogen, phosphorus, potassium, organic matter and lime contents following Richards (1954). All the results of the field and subsequent laboratory experiments were statistically analyzed using Z and t tests.

Effect of Aqueous Extract. Leaves, stems, fruits and barks of walnut were collected and dried at room temperature (25-30℃). Fresh material was kept at 5-10℃. Glassware was sterilized at 170℃ for at least 4 hours while heat labile substance were autoclaved at 115 lbs. Pressure at 110℃ for 30 minutes.

Five gm of crushed fresh or dried leaves, stems, fruit-epicarps and barks were soaked in 100 *ml* distilled water for 24 hours at 25℃ and filtered. These extracts were stored at 5-10℃ until they were used. However, they were generally utilized within a week. Ten seeds of corn (*Zea mays* cv. Sarhad White), Turnip (*Brassica campestris* Linn. subsp. *rapa*) and Bean (*Phaseolus vulgaris* Linn.) were placed in petri dishes on three folds of Whatman No. 1 filter paper seed-beds. The dishes were moistened with another single sheet of filter paper. Distilled water was used as a control. There were 10 replicates for each species in every treatment. The dishes were incubated at 25℃. Germination, radicle, plumule growth and number of seminal roots (for corn and bean) were determined after 72 hours.

Effect of Litter. One gm dried leaves, freshly fallen leaves and fruit epicarps were spread uniformly in a petri dish and topped with a single sheet of filter paper. Control was made similarly by replacing litter with pieces of filter papers. Equal amount of distilled water was added to every dish. After 4 hours, seeds of the above mentioned test species were placed on the top of filter papers and incubated as before. At the end same parameters were measured. There were 10 replicates, each with 10 seeds.

Effect of Natural Rain Leachate. Five hundred gm of crushed walnut shoots (leaves and stems) were taken in triplicate over a sheet of filter paper in large glass funnels. These funnels along with stands were placed on a 1 meter high bench during slow drizzle. A flask, kept beneath every funnel, received the rain leaching through the shoots. The direct entry of rain water was prevented into these flasks. Direct rain water was simultaneously collected for making control. After 4 hours drizzle enough leachate was collected and the leachates from three flasks mixed together. A portion of this rain leachate was concentrated to two (2 ×) and four (4 ×) times the original concentration (1 ×). These leachates along with rain water were used against the same three crop species as in the aqueous extract bioassay. Another portion of rain leachate was saved for chromatographic analysis.

Effect of Walnut-affected Soil. Soil with or without walnut influence was collected from top 15 cm depth. It was dried, sieved, through 2 mm mesh and used in soil extract and soil bed bioassays following our standard technique (Hussain *et al.*, 1979; Hussain, 1980; Hussain and Gadoon, 1981). Germination, radicle, plumule growth and number of seminal roots were measured. Ten seedlings were randomly selected from each of the 10 replicates for the determination of fresh and dry weight. The seedlings were oven dried at 65℃ for 72 hours. Moisture contents were calculated on oven dry basis.

Table 1. Physico-chemical analysis of the soils from four crop species with and without walnut influence. Each value is a mean of 10 fields with duplicate samples from each condition

Test crop	Soil	pH	Conductivity dSm^{-1}	Total soluble salts (%)	N (%)	P (ppm)	K (ppm)	OM (%)	Lime (%)
Corn	A	8.0	0.20	0.064	0.145	29.9	546	2.90	2.50
	B	8.1	0.25	1.081	0.140	17.3	490	2.15	2.18
Potato	A	7.7	0.60	0.192	0.112	25.1	163	2.24	2.25
	B	8.1	0.18	0.158	0.094	18.5	173	1.89	2.00
Turnip	A	7.8	0.33	0.067	0.120	24.0	236	5.13	2.00
	B	7.7	0.27	0.069	0.117	25.1	230	4.69	2.21
Bean	A	8.2	0.32	0.128	0.240	27.5	200	4.33	2.00
	B	8.0	0.29	0.120	0.242	26.9	211	4.23	2.15

A means sandy loam collected under the walnut tree stand.
B means sandy loam collected out of the walnut tree stand.

Table 2. The growth performance of corn at 2 different localities under the influence of walnut

Habitat	Shoot length (cm)	No. of leaves	Shoot fresh weight (gm)	Shoot dry weight (gm)	Ear length (cm)	No. of grains/Ear	100 grain weight (gm)
	a. Kawai, July 15, 1987						
Under walnut	22.5	3.50	4.36	1.11	-	-	-
Without walnut	33.8	5.70*	11.80*	3.19*	-	-	-
	b. Kalam, July 7, 1987						
Under walnut	8.70	7.2	12.56	5.11	-	-	-
Without walnut	23.00*	11*	79.95**	34.01*	-	-	-
	c. Kalam, October 10, 1987						
Under walnut	52.11	6	25.33	9.33	11.7	84	79
Without walnut	153.67**	10	168.00**	16.08*	22.5*	250**	150*

Each value is a mean of 10 fields, each with 20 plants.
* and **=Significantly different from control at $p=0.05$ and 0.01, respectively.

Identification of Phytotoxins. Ten percent aqueous extract and rain leachate of walnut were concentrated to 1/3 of its original volumn and acidified to pH 2.5 with in HCl. It was extracted three times with sufficient amount of ether. The three ether fractions were mixed and concentrated in rotavapor under reduced pressure. The concentrate was finally dried. The aqueous fractions were also concentrated to almost 10 *ml*. Whatman No. 1 chromatographic paper was spotted with the concentrated aqueous portion and ether residue dissolved in 2 *ml* ethanol. The procedures used to isolate the compounds from the above mentioned materials were those of Naqvi (1976) and Lodhi and Rice (1971). Standard compounds were run simultaneously for comparison. Rf, colour with the spraying reagents and UV light was recorded to identify the phytotoxins.

RESULTS

Field Observations. The analysis of soils indicated no significant difference in the texture and nutrient status of soils from places with or without the influence of walnut for each of the crop species (Table 1).

The growth, fresh and dry mass of corn plants were significantly less under the influence of walnut than their counter parts in the same field without the influence of walnut at both the observations and localities (Table 2). Similarly, the ear length, number of grains/ear and

Table 3. Effect of walnut on the growth performance of three crop species in association with walnut

Parameters	Without walnut (control)	Under Walnut	% of control
a. *Potato*			
1. Shoot length (cm)	60	31*	51.67
2. Shoot fresh weight (gm)	84.10	20.8**	24.73
3. Shoot dry weight (gm)	36	14**	38.89
4. No. of tubers	15	5*	33.33
5. Fresh weight of tubers (gm)	47	5**	10.64
b. *Turnip*			
1. Shoot length (cm)	24.22	20.11*	83.03
2. Shoot fresh weight (gm)	140	93.6*	66.86
3. Shoot dry weight (gm)	29	14**	48.28
4. No. of leaves	7	6*	85.71
5. Fresh weight of tubers (gm)	26	18*	69.23
c. *Bean*			
1. Shoot length (cm)	108	62*	57.41
2. Shoot fresh weight (gm)	223	52**	23.32
3. Shoot dry weight (gm)	75	10**	13.33
4. No. of pods/plant	12	4**	33.33
5. Length of pod (cm)	13	8**	61.54
6. Grains/pod	5	8.28*	65.6
7. Fresh weight of pods (gm)	27	13**	48.15
8. Dry weight of pods (gm)	6.50	2.31**	35.54
9. 100 grains weight (gm)	95	63*	66.32

Each value is a mean of 10 fields, each with 20 plants (October 10, 1987) Kalam District Swat.

* and **=Significantly different from control at p=0.05 and 0.01, respectively.

weight of grains were also significantly lower in the walnut influenced part of the fields.

The shoot growth of potato, turnip and bean was poor under walnut influence (Table 3). The number and weight of potato tubers, weight of turnips; length, number of pods, grains/pod and weight of grains of beans significantly reduced under walnut canopy (Table 3).

In all these cases the soil nutrients were seemingly similar in the same crop field yet growth suppression was obvious in each of crops.

Effect of Aqueous Extracts. Excepting the germination of turnip and beans in fresh and dried stem extracts, all the test species exhibited significantly retarded germination in the various treatments (Table 4). The number of seminal roots (corn and bean) in all the treatments reduced in the various extracts (Table 4). However, corn in fresh epicarps and bean in fresh stems, fresh and dried epicarps remained unaffected.

The radicle growth of three test species decreased significantly in all the test conditions (Table 4). However, the radicle growth of bean in dried stems and fresh epicarps was not inhibited. Similarly, the plumule growth of turnips and beans remained unaffected in the fresh stems. In the remaining cases the plumule growth of all test species got suppressed (Table 4).

Effect of Litter. The percent germination, number of seminal roots, radicle and plumule growth of all the test species declined when grown on litter beds (Table 5). However, the germination of bean in dried leaf litter and plumule growth of turnip in the fresh leaf litter was

Table 4. Effect of aqueous extracts of various parts of walnut tree on the germination (% of control) and early seedling growth (% of control) of three crop species

Item	Test crop	Walnut extracts						
		Fresh leaves	Dried leaves	Fresh stems	Dried stems	Fresh epicarps	Dried epicarps	Barks
Germination	Corn	76.08	78.26*	84.78*	86.96*	78.26*	69.57*	65.22*
	Turnip	53.66**	31.71**	95.12	100.00	78.05*	70.73*	36.59**
	Bean	81.40*	86.05*	95.35	97.67	83.72*	86.05*	74.42*
No. of seminal roots	Corn	56.72**	56.14**	74.85*	76.60*	89.47	42.69**	53.22**
	Bean	72.07*	84.52*	92.83	80.37*	93.40	95.47	19.25**
Radicle growth	Corn	43.49**	28.00**	62.18**	80.82*	52.56**	24.58**	26.25**
	Turnip	11.96**	7.54**	63.19*	83.60*	31.88**	12.86**	12.66**
	Bean	40.26**	41.55**	79.45*	90.91	80.01**	86.10*	15.16**
Plumule growth	Corn	19.90**	15.81**	58.41**	64.40**	62.59*	42.60*	21.99**
	Turnip	31.86**	15.68**	91.33	86.44*	78.43*	23.53*	17.40**
	Bean	46.31*	60.30*	88.94*	77.78*	62.38*	64.74*	22.78**

Each value is a mean of 10 replicates, each with 10 seeds.

* and **=Significantly different from control at p=0.05 and 0.01, respectively.

Table 5. Effect of walnut litter on the germination (% of control) and early seedling growth (% of control) of three crop species

Item	Test crop	Walnut extracts		
		Fresh leaves	Dried leaves	Fresh epicarps
Germination	Corn	73.91*	78.26*	78.26*
	Turnip	75.61*	51.21**	65.85*
	Bean	74.42*	88.37*	69.77*
No. of seminal roots	Corn	61.98*	62.57*	73.68*
	Bean	66.03*	66.04*	77.36*
Radicle growth	Corn	54.77*	26.86**	59.64*
	Turnip	61.06*	16.96*	61.93*
	Bean	70.38*	28.21**	39.55**
Plumule growth	Corn	43.25**	23.46**	54.84**
	Turnip	92.32	17.48**	81.05*
	Bean	56.52*	61.24**	70.84*

Each value is a mean of 10 replicates, each with 10 seeds.
* and ** = Significantly different from control at p=0.05 and 0.01, respectively.

Table 6. Effect of natural rain leachates from walnut shoots on the germination (% of control) and seedling growth (% of control) of three crop species

Item	Test crop	Concentrated rain leachate		
		1 ×	2 ×	4 ×
Germination	Corn	119.51	87.80*	65.85*
	Turnip	95.23	71.43*	50.00**
	Bean	97.82	89.13	72.83*
No. of seminal roots	Corn	113.88	81.84*	73.06*
	Bean	109.93	70.57*	48.94**
Radicle growth	Corn	129.68	88.90	66.71*
	Turnip	72.97	28.28**	19.52**
	Bean	109.54	87.47*	77.11*
Plumule growth	Corn	143.40	61.84	36.99**
	Turnip	102.53	64.92*	38.17**
	Bean	53.45	50.29**	25.68**

Each value is a mean of 10 replicates, each with 10 seeds.
* and ** = Significantly different from control at p=0.05 and 0.01, respectively.

not affected.

Effect of Natural Rain Leachate. The germination of all the test species were significantly decreased in the concentrated rain leachate for four times (4 ×). While 1 × and 2 × treatments almost had no effect on the germination with the exception of turnip in 2 × rain leachate. The number of seminal roots. Radicle and plumule growth of all the test species significantly dwindled in 2 × and 4 × rain leachates. However, radicle growth of corn in 2 × was the exception (Table 6). Turnip seedling also exhibited reduced growth in 1 × treatments.

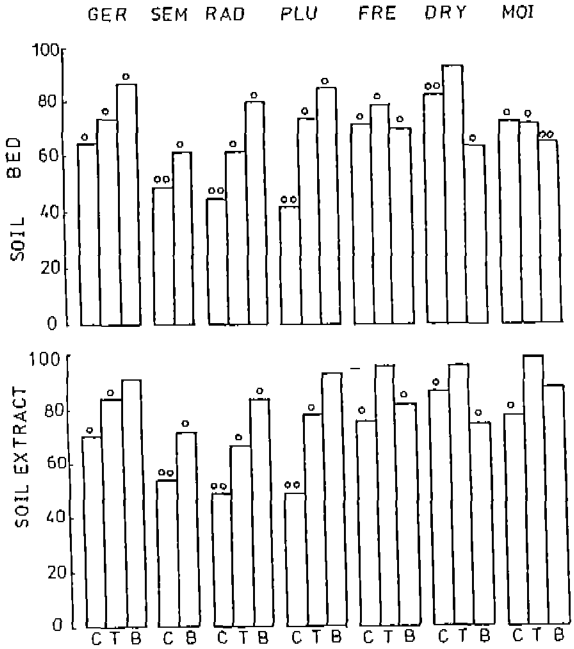

Fig. 1. Effect of walnut affected soil on the germination and seedling growth of corn (C), turnip (T) and bean (B). Data represented is percent of control and each one was a mean of 10 replicates. ∘ and ∘∘ mean significantly different from control at p=0.05 and 0.01, respectively. GER, germination; SEM, seminal root; RAD, radicle growth; PLU, plumule growth; FRE, fresh weight; DRY, dry weight; MOI, moisture contents.

Effect of Walnut-affected Soil. Excepting the germination of bean in soil extract, the remaining test species exhibited retarded germination in the walnut-affected soil (Fig. 1). The number of seminal roots, radicle and plumule growth of all the test species, except bean in soil extracts, had poor seedling growth. The fresh and dry weight and moisture contents of all the three test species, except fresh and dry weight of turnip and moisture contents of bean in soil extract, severely decreased in the walnut affected soil.

Identification of Phytotoxins. Some of the phytotoxins identified from aqueous extracts and rain leachates of walnut shoots were ferulic, *p*-coumaric, caffeic, vanillic, *p*-hydroxybenzoic, chlorogenic and gallic acid (Table 7). These are water soluble with allelopathic behaviour in

Table 7. Chromatographic analysis of phytotoxins from rain leachates and aqueous extracts of walnut

No. Compounds	Rf on Whatman No. 1		Fluorescence		Reagent colours		
	6% AA	BAW	Long	Short	SA	FC	PN
1. Ferulic acid	.40	.88	b.blue	b.blue	f.tan	blue	f.brown black
Suspected ferulic acid	.39	.87	b.blue	b.blue	f.tan	blue	f.brown black
2. *p*-Coumaric acid	.47, .70	.90	p.abs	p.abs	o.red	blue	brown black
Suspected *p*-coumaric acid	.46, .71	.89	p.abs	p.abs	o.red	blue	brown black
3. Caffeic acid	.32, .66	.80	blue	blue	none	blue	f.brown black
Suspected caffeic acid	.33, .67	.81	blue	blue	none	blue	f.brown black
4. Vanillic acid	.56	.92	f.abs	p.abs	light orange	blue	purple
Suspected vanillic acid	.57	.91	f.abs	p.abs	light orange	blue	purple
5. *p*-oh-benzoic acid	.63	.91	f.abs	p.abs	o.red	blue	f.wine
Suspected *p*-oh-benzoic acid	.64	.90	f.abs	p.abs	o.red	blue	f.wine
6. Chlorogenic aicd	.57, .71	.65	blue	blue	tan	blue	brown
Suspected chlorogenic acid	.58, .70	.66	blue	blue	tan	blue	brown
7. Gallic acid	.44	.65	none	b.lav	f.tan	blue	f.brown black
Suspected gallic acid	.45	.65	none	b.lav	f.tan	blue	f.brown black

See text for solvent systems.
SA, diazotized sulfanillic acid; FC, ferric chloride-potassium ferricyanide; PN, diazotized *p*-nitroaniline.
b, bright; f, faint; abs, absorption; o, orange; lav, lavender.

many other plants also (Lodhi and Rice, 1971; Lodhi, 1975; Naqvi, 1976; Kil and Yim, 1983; Rice, 1984; Ayaz *et al.*, 1989).

DISCUSSION

There were not much differences in the physical and nutrient status of the soils taken from beneath and away from the walnut trees with in the same field. Yet, reduction in the growth parameters and yield of the associated crops like potato, corn, turnip and bean were much obvious under the influence of walnut trees compared with their counter parts growing away from walnut in the same field. Such significant reduction in the growth under seemingly similar habitat conditions are attributable to allelopathy. Lodhi and Rice (1971) observed reduced growth of associated species under *Celtis laevigata*. Kil and Yim (1983) also reported the suppression of herbaceous species by *Pinus densiflora* due to allelopathy. And Hussain *et al.* (1985) also support our findings who observed poor crop growth underneath *Melia azedarach* trees. The present findings also agree with those of Davis (1928) and Massey (1925) who reported bare species under walnuts. Banerji (1981) also attributed the poor growth of associated species due to phytotoxicity of walnut. Many woody species retard the germination and seedling growth allelopathically (Lodhi and Rice, 1971; Kil and Yim, 1983; Rice, 1984; Putnam and Tang, 1986; Waller, 1987) and our findings agree with them. Fisher (1987) and Putnam and Tang (1986), who reported similar allelopathic behaviour of other walnut species, also strengthen our results. The present findings also receive support from Roschina (1973), Ponder (1981) and Putnam and Weston (1986) who suspected *Juglans regia* to be allelopathic. Manifest of allelopathy by aqueous extracts from woody species is well documented in literature.

The phytotoxicity of walnut was part specific as leaves were more inhibitory than other parts. Lodhi and Rice (1971), Quinn (1974), Hussain *et al.* (1979), Datta and Chatterjee (1980) and Kil and Yim (1983) also reported differential toxicity of plant parts and varied response of the test species used. The aqueous extracts contain water leachable phytotoxins. Rain leachates from walnut were demonstrably deleterious to the crop species owing to phytotoxins. Ayaz *et al.* (1989) showed that rain leachates from *Adhatoda* contained several inhibitors. Kil and Yim (1983) and Lodhi and Rice (1971) also demonstrated rain leachates of red pine to be allelopathic. Stickney and Hoy (1981) reported poisonous drip from black walnut causing growth suppression. We beleive that phytotoxins in the rain leachates, fog/dew drips and stem flows are released by natural water from walnut. These substances subsequently accumulate in the soil to render it unfavourable for the accompanying susceptible species. Walnut-affected soils when used as a growth medium for the test crop species prevented their germination and reduced seedling growth. Although, the nutrient status of both walnut-affected and unaffected soils was almost similar. Yet, it was inhibitory owing to phytotoxins received from walnut. Soil-plant phytotoxicity by other species has been reported by many workers (Wang *et al.*, 1976; Lodhi and Rice, 1971; Lodhi, 1975; Rice 1984). The present results are supported by Horsley (1976), Kil and Yim (1983), Hussain *et al.* (1985), Putnam and Tang (1986) and Waller (1987) who reported soil-plant phytotoxicity of many woody species. The soil underneath walnut might also intoxicate due to toxic root exudates as reported by Massey (1925). The test seedlings had reduced biomass and moisture contents.

Similar findings have been reported by Lodhi and Nickell (1973), Dirvi and Hussain (1979) and Rice (1984).

Many allelopathic compounds including those identified in the present study have been isolated from soils underneath allelopathic plants. The present findings suggest that walnut releases inhibitors by natural and/or irrigation waters and deposited in the immediate soil to affect the co-occurring species. The creation of bare spaces and poor growth of crops under walnut canopy is mainly due to allelopathy. The climatic conditions in Kalam are of dry temperate type having low summer rainfall and heavy snow fall during winter. The litter deposited in the autumn remains mostly undecayed during winter owing to snow cover. It starts decaying during spring/summer which receives poor rains. Low rain fall helps retaining the phytotoxins and intensifies the allelopathic stress. The amount and time of rain fall plays an important role in the manifest of allelopathy. Ecologically speaking walnut is strongly allelopathic. Yet, it is cultivated on agricultural lands as it out-weights its negative effects by being a source of cash income to the inhabitants.

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적 요

Juglans regia L. 나무밑에는 다른 식물이 잘 자라지 못하고 있다. 그래서 그 임상토양, 수용추출액, 빗물 등을 써서 작물을 심어 본 결과 발아와 생장이 심하게 억제되었다.

그 억제물질을 찾기 위해서 *Juglans regia* L. 나무의 수용추출액과 빗물을 종이크로마토그래피법으로 분석하여 ferulic acid, *p*-coumaric acid, caffeic acid, vanillic acid, *p*-hydroxybenzoic acid, chlorogenic acid, gallic acid를 분리 확인했으며, 이 물질들이 억제작용에 관계한 것으로 추정한다.

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