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ALLELOPATHIC POTENTIAL OF TREE OF HEAVEN (*AILANTHUS ALTISSIMA* SWINGLE)

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ABSTRACT

Aqueous extracts of *Ailanthus altissima* Swingle leaflets, rachises and roots reduced radicle growth of garden cress. The growth inhibitory activities of aqueous root extracts were significantly higher than that of the leaflets and rachises aqueous extracts. Methanol extracts of leaflets were more toxic than the aqueous extracts, whereas the chloroform extracts exhibited very low inhibitory activities. The aqueous, methanol and chloroform extracts of leaflets at 50 g l^{-1} inhibited growth by 63.1, 90.6 and 9.2%, respectively. In contrast, the chloroform extracts of rachises significantly reduced the radicle length in garden cress. At 50 g l^{-1}, the aqueous, methanol and chloroform extracts of rachises inhibited growth by 61.6, 90.2 and 84.3%, respectively. The *Ailanthus* leaflets and rachises had the highest seasonal activity in spring and early autumn such that phytotoxin synthesis apparently follows optimum temperatures for growth. Post-emergence applications of aqueous extracts of leaflets on garden cress, pigweed, barnyard grass and maize exhibited broad-spectrum herbicidal activities by reducing growth to 58-71% of the control. Aqueous extracts of leaflets and rachises significantly reduced peroxidase activity by up to 85% and respiration (O_2 uptake) in root pieces by up to 66% in all plant species tested. *Ailanthus* tissues aqueous extracts also decreased mitosis in onion

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root tips. In the presence of 50 g l^{-1} of root extract, 90% reduction in mitosis was observed. These biochemical and physiological changes are probably among the various cellular targets for *Ailanthus* allelochemicals responsible for growth inhibition in higher plants.

Key words: *Ailanthus altissima* Swingle , Allelochemicals, Garden cress, Mitosis, Peroxidase phytotoxicity, Tissue extracts, Respiration.

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پتانسیل اللو پاتیک درخت پردیس (*Ailanthus altissima* Swingle)

علی مرادشاهی، پونه یغمائی و حسین غدیری

به ترتیب استادیار و دانشجوی سابق کارشناسی ارشد بخش زیست شناسی دانشکده علوم و دانشیار بخش زراعت دانشکده کشاورزی دانشگاه شیراز، شیراز، جمهوری اسلامی ایران.

چکیده

عصارهٔ آبی برگچه، برگاسه و ریشه درخت پردیس (*Ailanthus altissima* Swingle) رشد طولی ریشه چهٔ گیاه ترتیزک را کاهش داد. اثر عصارهٔ متانولی برگچه بر رشد ریشه چهٔ ترتیزک همانند عصاره آبی بود اما اثر بازدارندگی عصارهٔ کلروفرمی بسیار کم بود که نشان می دهد آللوکمیکال های برگچه درخت پردیس نسبتا قطبی هستند. عصاره های آبی ، کلروفرمی و متانولی برگاسه دارای اثر بازدارندگی زیاد و همانند بودند که نشان دهندهٔ حضور آللوکمیکال های قطبی و غیر قطبی در برگاسه درخت پردیس است. بررسی تغییرات فصلی آللوکمیکال های درخت پردیس نشان داد که در بهار و اوائل پاییز اثر بازدارندگی عصاره های آبی و برگاسه بیشترین بود. هنگامی که اثر عصارهٔ آبی برگچه به صورت تیمار پس رویشی بر گیاهان ترتیزک ، بژگال، تاج خروس و ذرت مورد آزمایش قرار گرفت مشخص گردید که عصارهٔ فوق دارای خاصیت علف کشی قوی بر گیاهان تک لپه و دو لپه می باشد. در حضور عصاره های آبی برگچه و برگاسه درخت پردیس

فعالیت آنزیم پراکسیداز و میزان تنفس در تمام گیاهان مورد آزمایش کاهش یافته و همچنین تقسیم میتوز در ناحیه مریستمی ریشهٔ پیاز کاهش نشان داد. تغییرات بیوشیمیائی و فیزیولوژیک مشاهده گردیده احتمالا جزئی از هدف های سلولی آللوکمیکال های درخت پردیس می باشند که از آن طریق کاهش رشد در گیاهان عالی را سبب می گردند.

INTRODUCTION

Tree-of-heaven (*Ailanthus altissima* Swingle) is a fast growing and extremely aggressive tree which rapidly invades and colonizes new habitats. It has been shown that *A. altissima* produces compounds that are toxic to other plants (6, 9, 12). Allelopathy caused by these biochemicals is thought to contribute to the aggressive nature of *A. altissima* (7). Heisey and Delwiche showed that germination and growth of some plant species are inhibited by aqueous extracts of *Ailanthus* foliage (9). Heisey reported the presence of toxic substances in *Ailanthus* foliage and in bark of roots and the trunk. Crude extract of root bark showed strong herbicidal activity on redroot pigweed (*Amaranthus retroflexus* L.), velvetleaf (*Abutilon theophrasti* Medic.), foxtail (*Setaria glauca* [L.] Beauv.) and barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) (6). *A. altissima* tissues also have insecticidal, nematocidal and antifeedant activity and have been used in China to control agricultural pests (5,17,22).

Some investigators (8, 14) have reported isolation and identification of allelochemicals from *A. altissima* tissues. Lin *et al.* (14) reported the isolation and identification of ailanthone as the active ingredient in the stem bark of *A. altissima*. One year later, in 1996 , Heisey isolated ailanthone from root bark and showed that it has strong broad-spectrum herbicidal activity (8). Ailanthone is currently undergoing various biological tests to evaluate its potential for development as a natural-product herbicide. Callus cultures derived from *A. altissima* tissues have been examined for ailanthone production (10). Addition of indole-3-acetic acid (IAA) to the culture medium induced production of ailanthone by the calli.

Limited information is available on the physiological and biochemical effects of *A. altissima* extracts on other plant species. The present paper describes the seasonal changes in toxicity of extracts from *A. altissima* tissues, and their effects on germination and seedling growth, cell division, peroxidase activity and respiration of garden cress, pigweed, barnyard grass and onion.

MATERIALS AND METHODS

Plant Materials

A. altissima tissues were collected from trees at Eram botanical garden, Shiraz University. Pigweed (*Amaranthus retroflexus* L.) and barnyard grass (*Echinochloa crus-galli* L.) seeds were collected from natural stands at the College of Agriculture, Shiraz University, and seeds of garden cress (*Lepidium sativum* L.) were purchased from a local market.

Tissue Extract Preparation

Leaflets, rachises and roots were rinsed with deionized water and cut into small pieces. Extracts were prepared by homogenizing 10 g of fresh tissue in 100 ml of deionized water, methanol or chloroform with a Warring blender. The homogenates were stirred at room temperature for 2 hr on a magnetic stirrer and then centrifuged at 6000 g for 15 min. The supernatants was decanted and the methanol and chloroform extracts were placed under a stream of N_2 at room temperature to evaporate the solvent. The residue was reconstituted to 100 ml with deionized water.

Bioassay Procedure

Due to the sensitivity of garden cress to phytotoxins, bioassays were performed on seeds of garden cress. The seeds were soaked for 10 min in 1% sodium hypochlorite and thoroughly rinsed with deionized water. Ten seeds were placed on filter paper in 9×1.5-cm petri dishes and 5 ml of the original or diluted extracts was added to each petri dish. The dishes were incubated in darkness at 24±4°C. Radicle and plumule lengths were measured after 3 d and used as an index of allelochemical activity of extracts. Control dishes contained 5 ml deionized water. There were three dishes per extract and each experiment was replicated three times.

Seasonal Variations in Toxicity

Seasonal variations in toxicity were determined as above except that the aqueous extracts were prepared from tissues sampled monthly for 8 successive months during the growing season of *A. altissima* trees. The same trees were sampled on all collection dates from April to November 2000.

Sampling younger true leaves at the top of the branches minimized variability between leaves of different ages.

Herbicidal Potential of *A. altissima*

Two sets of experiments were conducted to test the herbicidal potential of *A. altissima* allelochemicals. In one set of experiment, seeds of pigweed, barnyard grass and water cress were treated as in the bioassay procedure described above. Radicle and plumule lengths were determined after 3 d and compared with controls. In another set of experiments, the extracts were applied post-emergence to seedlings growing in 19×13×4-cm flats containing 450 g sterile farm soil. For postemergence treatments, the flats were sown with 300 mg of cress, pigweed or barnyard grass seeds and kept in a growth room set at 24±4° C and a 16-hr photoperiod. Flats were sprayed with 50 ml of extracts 7 d after planting by which time most seedlings had emerged. Shoots were harvested at soil level 7 d after spraying, washed and then dried at 50° C for 48 hr and weighed. Control flats were sprayed with deionized water and each experiment was replicated three times.

Extraction and Measurement of Peroxidase Activity

Total peroxidase was extracted from roots of 10 to 15-d-old cress, pigweed and barnyard grass and assayed according to MacAdam *et al.* (16). To determine the effects of *A. altissima* allelochemicals on peroxidase activity, the reaction mixtures in a final volume of 3 ml contained 1.5 ml of leaflet or rachis aqueous extracts. Changes in absorbance at 436 nm were recorded at 15-sec intervals. The slopes of the lines were calculated and the activities were expressed as percentage of the control.

Cell Division

The effects of *A. altissima* extracts on mitosis were determined using onion (*Allium cepa* L.) bulbs rooted in water in the laboratory. Root tips, 2 cm in length, were cut from the bulbs and incubated with 25, 50 and 100 g l^{-1} of extracts at room temperature. After 4 hr, the root tips were stained with fulgen and used to determine the percentage of mitotic cells and compared to the water control (19).

Respiration

The standard Warburg manometric apparatus was used to determine the effects of extracts on respiration. Roots of cress, pigweed and barnyard grass, about 2 cm long, were cut into 0.5 cm pieces and incubated in Warburg flasks with 1.5 ml of 50 mM phosphate buffer pH=5.5, and 1.5 ml of aqueous extracts. Control flasks contained 3 ml of phosphate buffer. Changes in the height of colored liquid in the barometers were recorded and used to calculate O_2 consumption by root pieces.

RESULTS AND DISCUSSION

Seasonal variations of toxicity of *A. altissima* leaflets and rachises are shown in Fig. 1. Aqueous extracts from tissues sampled in May reduced radicle length of garden cress by 82% relative to the control. This suggests the presence of a high concentration of toxic compounds in the tissues. Reduced radicle growth also occurred with extracts of tissues sampled in September that indicates a second peak in allelochemical biosynthesis. The toxicity of leaflet extracts seems to be higher than rachis extracts.

Seasonal variations of juglone in black walnut leaves, sapsapo in holly leaves and toxic substances in *A. altissima* tissues have been reported by other investigators (3, 6, 18). Heisey (6) showed that *A. altissima* leaflets were most toxic after emergence in spring but the second peak in toxicity, in contrast with the present research, was not observed by Heisey that may be due to difference in environmental conditions in New York and Shiraz.

Low concentrations of *A. altissima* aqueous extracts of roots reduced radicle length of garden cress significantly (Table 1). Aqueous extracts of roots, rachises and leaflets at 50 g l^{-1} caused 92, 64 and 59% reduction in radicle length, respectively. Similar results were obtained when pigweed and barnyard grass were treated with the same extracts (data not shown). When compared with the effects of aqueous extracts, the methanol extract of leaflets had a greater phytotoxic activity and the chloroform extract had less inhibitory effects (Table 2). In fact, there was some promotion of growth by the leaflet chloroform extract at 10 g l^{-1} The methanol, aqueous and chloroform extracts of leaflets at 50 g l^{-1} inhibited radicle growth by 90.6, 63.1 and 9.2%, respectively. In contrast, the inhibitory effects of both chloroform and methanol extracts of rachises were higher than the inhibition caused by water extract (Table 3). At 50 g l^{-1} the methanol, aqueous and chloroform extracts of rachises reduced radicle length by 90.2, 61.6 and 84.3%, respectively. Consistent and similar effects of aqueous, methanol and chloroform extracts of leaflets and rachises were observed on radicle elongation of pigweed and barnyard grass (data not shown).

Table 1. Effects of aqueous extracts of *A. altissima* root, rachis and leaflet on radicle length (mm) of garden cress.

Extract	Length at indicated concentration (g l^{-1})				
	0	0.1	10	50	100
Root	20.3Aa†	9.2B	2.7Cb	1.7Cb	-
Rachis	21.4A	-	17.4Ba	7.6Ca	5.9Ca
Leaflet	21.1Aa	-	17.9Ba	8.7Ca	3.3Db

† Means with similar letters in each row (capital letters) and in each column (small letters) are not significantly different (Duncan 5%).

Table 2. Concentration effects of water, methanol and chloroform extracts of *A. altissima* leaflets on radicle length (mm) of garden cress. Values in parentheses are percent radicle length relative to the control.

Extract	Length at indicated concentration (g l^{-1})			
	0	10	50	100
Water	26.8Aa †	14.9Bb	9.9 Cb	5.5Cb
	(100)	(55.6)	(36.9)	(20.5)
Methanol	24.4 Aa	8.1Bc	2.3 Cc	2.1Cb
	(100)	(33.2)	(9.4)	(8.6)
Chloroform	21.7Aa	25.7Aa	19.7ABa	14.2Ba
	(100)	(118)	(90.8)	(65.4)

† Means with similar letters in each row (capital letters) and in each column (small letters) are not significantly different (Duncan 5%).

It is reported that the inhibitor(s) could readily be extracted from *A. altissima* leaflets and root bark with methanol but not dichloromethane, indicating the polar characteristics of allelochemical (s) (6). Similar results were obtained in the present research for leaflets, but the chloroform extract of rachises, reconstituted in water, had activity similar to the methanol extract and higher than the aqueous extract. It seams that in addition to polar allelochemicals, other phytotoxins extractable with chloroform, that are miscible and/or soluble in water, are also present in *A. altissima* rachises.

Table 3. Concentration effects of water, methanol and chloroform extracts of *A .altissima* rachises on radicle length (mm) of garden cress. Values in parentheses are percent radicle length relative to control.

Extract	Length at indicated concentration (g l^{-1})			
	0	10	50	100
Water	26.8Aa†	19.7Ba	10.3Ca	6.4Ca
	(100)	(73.5)	(38.4)	(23.9)
Methanol	24.4Aa	4.3 Bb	2.4 Bb	2.2Bb
	(100)	(17.6)	(9.8)	(9.1)
Chloroform	21.7Aa	15.0Ba	3.4 Cb	2.6Cb
	(100)	(69.1)	(15.7)	(11.9)

† Means with similar letters in each row (capital letters) and in each column (small letters) are not significantly different (Duncan 5%).

Aqueous extracts of leaflets exhibited herbicidal activity ($P< 0.05$) when sprayed on seedlings after they had emerged from the soil. Many seedlings were damaged and turned yellow within 6 d after spraying. The overall result of postemergence treatment was a 29, 32, 35 and 42% reduction in shoot dry weight in 7 d, for maize, cress, barnyard grass and pigweed, respectively.

The aqueous extracts of Ailanthus leaflets and rachises (Table 4) significantly reduced peroxidase activity in roots of garden cress, pigweed and barnyard grass. Several investigators (1, 21) have reported the effects of various compounds on peroxidase activity. Low concentration of cinnamic acid increased peroxidase activity in soybean roots whereas at higher concentrations, peroxidase was inactivated (1). Inhibition of peroxidase activity by para-coumaric acid has been reported in wheat roots (21).

A number of chemicals that have been implicated in allelopathy inhibit the activity of certain enzymes. cinnamic acid derivatives inhibit activity of phenylalanine ammonia-lyase (PAL). Several herbicides , such as glyphosate, and several microbial toxins studied for their herbicidal potential inhibit specific enzymes in biosynthetic pathways of amino acids (18, 20). Some heavy metals, such as cadmium, increase the activity of ionically bound peroxidase. The increased activity of this enzyme increases the cross-linkage of the polymers in cell walls and is partly responsible for root growth inhibition caused by cadmium (2).

Preliminary evaluation of the effects of *A. altissima* extracts on respiration showed that oxygen consumption by root pieces of garden cress, pigweed and barnyard grass was reduced when treated with aqueous extracts of *A. altissima* leaflets and rachises (Table 5). Respiration by root pieces of barnyard grass was less affected compared to the other plant species tested.

Table 4. Effects of aqueous extracts of *A. altissima* leaflets and rachises on peroxidase activity in roots of garden cress, pigweed and barnyard grass.

Extract	Leaflet extract (g l^{-1})				Rachis extract (g l^{-1})		
	0	10	50		0	10	50
				% of control			
Cress	100A†	20C	32B		100A	19B	21B
Pigweed	100A	31C	56B		100A	30B	15C
Barnyard grass	100A	88B	60C		100A	74B	34C

† Means with similar letters in each row within one extract are not significantly different (Duncan 5%).

Table 5. Effects of aqueous extracts of *A. altissima* leaflet and rachis on oxygen consumption by root pieces of garden cress, pigweed and barnyard grass.

Extract	Leaflet extract (g l^{-1})					Rachis extract (g l^{-1})			
	0	10	50	100		0	10	50	100
					% of control				
Garden-cress	100	90	81	51		100	84	71	54
Pigweed	100	59	54	52		100	86	54	34
Barnyard grass	100	81	81	74		100	100	71	84

Tests with mitochondrial suspensions have shown that allelochemicals from a variety of chemical classes inhibit mitochondrial O_2 uptake. Some allelochemicals such as juglone strongly inhibited the respiration (O_2 uptake) of bean and lettuce roots (13). Similar results were reported by Koeppe (11) using excised corn roots . At 500 μM, juglone inhibited O_2 uptake by more than 90 % after 1 hr exposure to the extract.

Aqueous extracts of *A. altissima* roots, leaflets and rachises reduced cell division in the root meristematic regions of onion (Fig. 2). The root extract inhibited mitosis severely; at 50 gl^{-1}, percent of cells in mitosis decreased from 11 to about 0.9%.

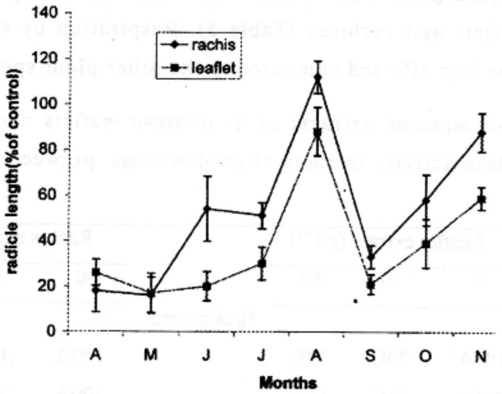

Fig. 1. Seasonal variations in toxicity of *A. altissima* leaflets and rachises bioassayed as aqueous extracts (50 g l^{-1}) on cress radicle growth. Each point represents the mean ± SE of 3 replicates.

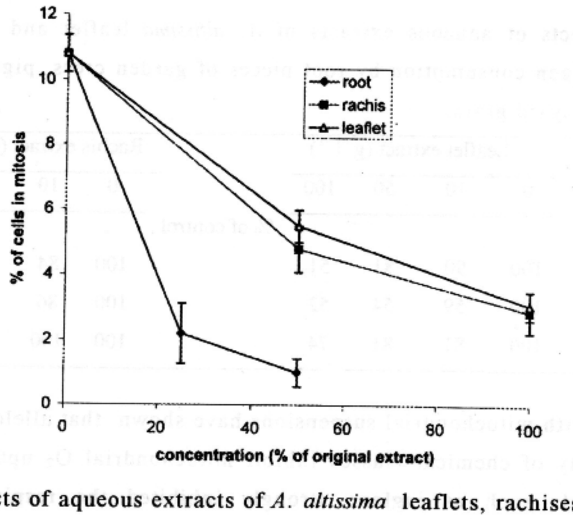

Fig. 2. Effects of aqueous extracts of *A. altissima* leaflets, rachises and roots on cell division in onion root tips. Each point represents the mean ± SE of 3 replicates.

Exposure of roots to volatile monoterpenes, primarily cineole and camphor were reported to cause reduced cell division and root cells with irregular nuclei. Coumarins and scopoletin decreased mitosis (4), benzylamine and hordenine caused disorganization of nuclei in linseed and white mustard root tips, respectively (15).

The present investigation demonstrates the occurrence of phytotoxins in *A. altissima* tissues that are active against a number of plant species. The cellular targets of allelochemicals that are responsible for growth inhibition have not been established for many compounds. We observed interference with respiratory oxygen consumption and as a result reduced ATP generation, impaired peroxidase activity and reduced cell division. These are probably among various cellular targets for *A. altissima* allelochemicals responsible for growth inhibition in higher plants.

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