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Preliminary Studies on Allelopatic Effect of Some Woody Plants on Seed Germination of Rye-Grass and Tall Fescue

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Abstract: In order to investigation of allelopathic effects of some ornamental trees on seed germination of rye-grass (*Lolium prenne*) and tall fescue (*Festuca arundinaceae*), this experiment was conducted in a randomized complete block design with 3 replicates at the laboratory of Horticultural Sciences Department of Ferdowsi University of Mashhad, during 2008. In this research, we studied the effect of aqueous and hydro-alcoholic extracts of Afghanistan pine (*Pinus eldarica*), arizona cypress (*Cupressus arizonica*), black locust (*Robinia psedue acacia*) and box elder (*Acer negundo*) leaves that prepared in 1:5 ratio on seed germination percent and rate for two grasses. The results showed that all extracts decreased statistically seed germination in compared to control treatment. The highest germination percentage and germination rate of tested grass detected in control treatment. Hydro-alcoholic extracts of all woody plants (15, 30%) were completely inhibited seed germination of rye-grass and tall fescue. Also aqueous extract of arizona cypress was completely inhibited seed germination of tall fescue and had more inhibitory activity than other aqueous extracts on rye-grass. Between aqueous extracts, the highest and lowest seed germination of rye-grass was found in Afghanistan pine and arizona cypress, respectively.

Key words: Afghanistan pine, arizona cypress, black-locust, box elder, aqueous and hydro-alcoholic extracts

INTRODUCTION

Allelopathy is an important mechanism of plant interference induced by the addition of plant-produced phytotoxins to the plant environment (Alam *et al*., 2001). Molisch (1937) coined the term allelopathy, referring to biochemical interactions between all types of plants, including microorganisms. Rice defined allelopathy as any direct or indirect, harmful or beneficial effect by one plant (including microorganisms) on another plant through production of chemical compounds that escape into the environment (Halbrendt, 1996; Rice, 1984). Bais *et al*. (2003) noted that in spite of classic opinion in which allelopathy refers to the chemical inhibition of one species by another, modern research suggested that allelopathic effects can be both positive and negative, depending upon the doses and organisms (Niakan and Saberi, 2009).

Allelochemicals are plant metabolites or their products that are released into the environment and may affect neighboring organisms by reducing cell membrane permeability, disrupting mineral uptake, or damaging genetic material (Mattner, 2001; Rizvi and Rizvi, 1992; Halbrendt, 1996). Allelopathic compounds or phytotoxins, escape into the environment by volatilization, decay of plant material and by exudation from roots (Alam *et al*., 2001). All plant parts including leaves, stems, pollen, flowers, roots, buds, rhizomes, seeds and fruits have been shown to contain allelochemicals, but leaves and roots are the most important source (Aldrich, 1984; Rizvi and Rizvi, 1992; Alam *et al*., 2001). Genetic characteristics and environmental conditions are the most effective agents in enhancing synthesis and exudation of allelochemicals (Pramanik *et al*., 2000). Chemicals that inhibit the growth of a species at a certain concentration may stimulate the growth of the same species or another at a lower concentration (Putnam and Tang, 1986). Therefore, the perceived ambiguous nature of allelopathy, caused that the phenomenon is sometimes hesitantly accepted, or even refuted, as an important factor in crop production (Rafiqul Hoque *et al*., 2003).

Commonly cited effects of allelopathy include reduced seed germination and seedling growth. Also delayed or inhibited germination and the stimulation or inhibition of root and shoot growth are often reported (Rizvi and Rizvi, 1992). Most of the growth and germination inhibitors produced by perennial angiosperms identified by Rice (1984) were derivatives of cinnamic acid or phenolic compounds. Also other authors found the inhibitory compounds including coumarins, flavonoids, alkaloids, cyanoglycosides, proteins and

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amino acids (Friedman and Waller, 1983; Waller, 1989; Dudai *et al*., 1999). To this list the terpenoid compounds must be added, including the volatile terpenes that are the main components of essential oils (Fischer, 1986; Muller, 1986; Elakovich, 1988).

Every tree manages the environment on under its canopy by physical or chemical means. Naturally the chemical influences appear stronger in tree species than in herbaceous species because of greater biomass in trees and longer consecutive inflows from trees compared to herbs (Ito and Ito, 2007). Allelopathy between agricultural crops and woody perennial has assumed a considerable importance for development of agroforestory system (Khan *et al*., 1999). One of the most famous allelopathic trees is Black Walnut (*Juglans nigra*). When certain other landscape plants are planted near or under this shade tree they tend to wilt, yellow and die. This decline occurs because the walnut tree produces a colorless, non-toxic, chemical called hydrojuglone that is oxidized into the allelochemical juglone, which is highly toxic when exposed to air or soil compounds. Tall fescue and Kentucky bluegrass grow better under walnut trees than other lawns except during drought conditions when soil moisture is low (Kocacaliskan and Teriz, 2001). Allelopathic effects have also been observed for other landscape trees including tree-of-heaven, sugar maple, hackberries, American sycamore, southern waxmyrtle, cottonwood, black cherry, red oak, black locust, sassafrass and American elm (Appleton *et al*., 2000). For example compounds inhibitory to the growth of neighboring plant species found in significant concentrations in the leaves and stems of young *Ailanthus altissima* ramets (Lawrence *et al*., 1991). Also in native stands of sycamore, there is a definite zone of reduced growth of associated species in areas of accumulation of fallen leaves and under the canopy. Leaf leachate, decaying leaves and soil collected under the canopy inhibited seed germination and growth of many associated species (Al-Naib and Rice, 1971). Several studies showed that large areas of the ground surface under the Eucalyptus remains completely bare. Alexander (1989) noted that the degradation of the soil under Eucalyptus showed the decrease in both base saturation and pH and the increase of cation exchange capacity (CEC) (El-Darier, 2002). The results of other study has shown germination of eastern hemlock and rosebay, also germination rates and radicle growth of lettuce, were inhibited by extracts of American chestnut leaves (Vandermast *et al*., 2002).

The accumulation and depletion of toxins in the soil is affected by factors such as soil type, aeration, drainage, temperature and microbial activity. For example coarse, well-drained, sandy soils would maximize leaching. By contrast, clay soils drain poorly and toxins do not leach readily, so trees on these soils may be severely damaged (Chick and Kielbaso, 1998).

Trees and turfgrass are commonly planted together in today's landscapes. However, they tend to be incompatible and interfere with one another, above ground and below. There have been numerous reports published in recent years on allelopathic effects between trees and grasses, but most of them have focused only on effects of grass on woody plants. We hypothesize that Afghanistan pine, arizona cypress, black locust and box elder leaf leachates have an allelopathic effect on associated grass species and this paper was aimed to identify the effects of these woody plants leaf extract on grass seed germination.

MATERIALS AND METHODS

Plant material: This research project was conducted from mid 2008 to early 2009. The leaves of afghanistan pine (*Pinus eldarica*), arizona cypress (*Cupressus arizonica*), black locust (*Robinia psedue acacia*) and box elder (*Acer negundo*) were used for preparation of the extracts. The experimental leaves of each species were collected freshly and dried at oven 50°C for 48 h. The seeds of *Lolium prenne* and *Festuca arundinaceae*, were obtained from Denmark.

Treatment: Maceration method by water or hydro-alcoholic solution (for 48 h) for preparation of extracts from leaves of woody plants was used. One gram of oven dried leaves was set in 5 mL of water or hydro-alcoholic solution (ethanol 15 and 30%). Dry residue (dry matter/ unit volume of extract) of the extracts was also determined (Table 1) as described by WHO (1984).

The seeds of *Lolium prenne* and *Festuca arundinaceae* were surface sterilized with 1% of sodium hypo-chlorite and then washed with distilled water three times. Germination (radicle emergence of 2 mm) tests were carried out in 9 cm Petri dishes at 24-28°C. Five milliliter of each extract was added to dishes containing 25 seeds of the test species sandwiched between two pieces of filter papers (Fujii *et al*., 2003). Water was used in control treatments. The number of seeds germinated in each container was recorded daily for one month. The statistical design was Randomized Complete Block Design (RCBD) with three replicates. Duncan's multiple range tests was used for comparing the means of seed germination percent and germination rate. Each petri dish was an experimental plot. Treatments were compared by applying Least Significant Difference (LSD) test at a 5% level of significance.

Table 1: The effect of some woody plants extracts on seed germination of *Lolium prenne* and *Festuca arundinaceae*

	Lolium prenne		*Festuca arundinaceae*		
Treatments	Germination (%)	Germination rate (seed day^{-1})	Germination (%)	Germination rate (seed day^{-1})	Dry residue
Aqueous extract of black locust (T_1)	46.67c	1.31c	28.00b	0.37b	2.0
Hydro-alcoholic extract of black locust 15% (T_2)	0.00e	0.00e	0.00c	0.00c	4.0
Hydro-alcoholic extract of black locust 30% (T_3)	0.00e	0.00e	0.00c	0.00c	3.5
Aqueous extract of box elder (T_4)	10.67d	0.59d	21.33b	0.36b	2.5
Hydro-alcoholic extract of box elder 15% (T_5)	0.00e	0.00e	0.00c	0.00c	3.5
Hydro-alcoholic extract of box elder 30% (T_6)	0.00e	0.00e	0.00c	0.00c	4.5
Aqueous extract of afghanistan pine (T_7)	69.33b	3.51b	26.67b	0.46b	2.0
Hydro-alcoholic extract of afghanistan pine 15% (T_8)	0.00e	0.00e	0.00c	0.00c	3.0
Hydro-alcoholic extract of afghanistan pine 30% (T_9)	0.00e	0.00e	0.00c	0.00c	3.0
Aqueous extract of arizona cypress (T_{10})	8.00d	0.41d	0.00c	0.00c	3.5
Hydro-alcoholic extract of arizona cypress 15% (T_{11})	0.00e	0.00e	0.00c	0.00c	5.0
Hydro-alcoholic extract of arizona cypress 30% (T_{12})	0.00e	0.00e	0.00c	0.00c	5.0
Control (T_{13})	84.00a	3.92a	64.00a	1.69a	
LSD 5%	6.48	0.36	11.77	0.31	

Values with different letters show significant difference

RESULTS AND DISCUSSION

The effect of extracts on seed germination of *Lolium prenne* and *Festuca arundinaceae* is shown in Table 1. The results show that all treatments had a significant inhibitory effect on seed germination of two grasses. Germination of lolium seeds completely inhibited by all hydro-alcoholic extracts. Likewise the aqueous extract of arizona cypress and all hydro-alcoholic extracts completely inhibited seed germination of *Festuca arundinaceae*.

The highest seed germination of lolium measured in control treatment. Aqueous extracts significantly decreased seed germination of lolium compare to control and aqueous extracts of afghanistan pine and arizona cypress have shown the highest and the lowest seed germination respectively (Table 1).

As shown in Table 1, there was a significant difference between aqueous and hydro-alcoholic extracts on lolium seed germination ($p<5\%$). There was no significant difference between aqueous extracts of box elder and arizona cypress for lolium percentage germination and germination rate, but a significant difference was found with other aqueous extracts. The Cumulative Percentage Germination (CPG) of *Lolium prenne* is shown in Fig. 1. On the 12th day of the control treatment almost 74% of the lolium seeds had germinated and after 4 weeks the control CPG was about 84%, but all hydro-alcoholic extracts completely inhibited seed germination of lolium. On the 28th day after treatment, 69% of lolium seeds that were treated with aqueous extract of afghanistan pine had germinated. This treatment had the lowest inhibitory effect on seed percentage germination.

Seeds of festuca in the control treatment showed the highest germination. Between the aqueous extract, the highest percentage germination of festuca measured in black locust and the lowest one in box elder extracts (28%, 21/33%), but there was not significant difference between them (Table 1). The Cumulative Percentage Germination (CPG) of *Festuca arundinaceae* is shown in Fig. 2. When leaf extracts was added to tall fescue seeds, cumulative percentage germination affected. On day 14, in the control plot near the 56% of festuca seed germinated and on the19th day the control CPG was 64%. On the 14th and 28th days after treatment, 13 and 24% of festuca seed that were treated with aqueous extract of afghanistan pine had germinated, respectively.

It is known some grasses besides some woody plants can be adversely affected by allelopathic effects. Allelochemicals are one of the most important components of the stresses that may influence seed germination, plant growth and nutrient uptake of the associated plant species (Rice, 1984). The present study indicates that the seed germination stage of selected grasses is decreased when exposed to different aqueous and hydro-alcoholic extracts of some woody plants. The reduction is more severe by hydro-alcoholic extracts. Dry residue determination of extracts also showed that all aqueous extracts contained lower dry residue than the hydro-alcoholic treatments (Table 1).

Allelopathic effects of tress have been little studied. Souto *et al*. (2001) showed the litter of *Pinus radiata* negatively affects seedling growth and seed germination of plants such as *Lactuca sativa* L., *Trifolium repens* L. and *Dactylis glomerata* L. Guerrero and Bustamante (2007) laboratory experiment revealed that germination of *Cryptocarya alba* seeds watered with an extract of leachate from *P. radiata* plantations was decreased compared to pure water, which suggests allelopathic effects of *P. radiata* litter. Also, Kato-Noguchi *et al*. (2009) reported that aqueous methanol extracts of red pine (*Pinus densiflora*) needles inhibited the growth of shoots and roots of cress (*Lepidium sativum*),

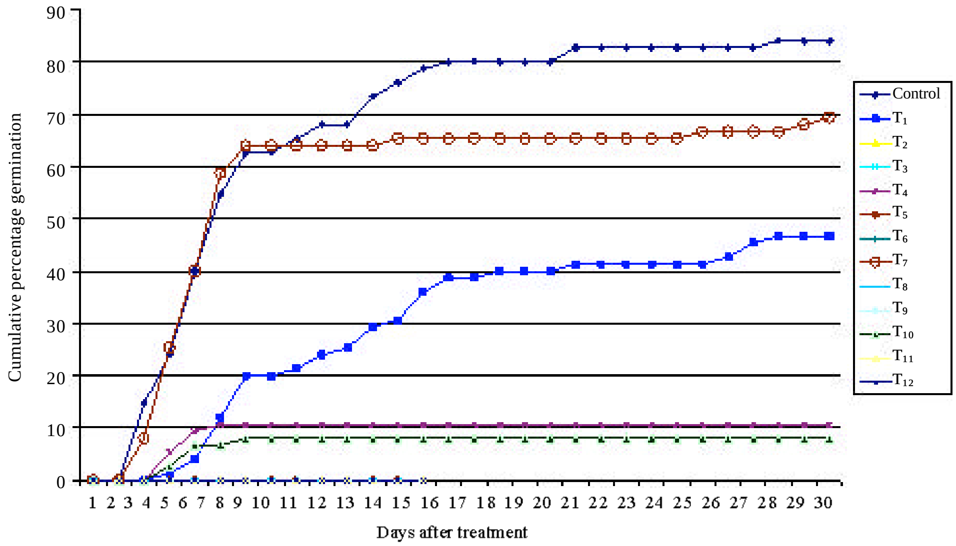

Fig. 1: Effect of different extracts on cumulative percentage germination of *Lolium prenne*. Treatments are as follow: T_1: Aqueous extract of black locust; T_2: Hydro-alcoholic extract of black locust 15%; T_3: Hydro-alcoholic extract of black locust 30%; T_4: Aqueous extract of box elder; T_5: Hydro-alcoholic extract of box elder 15%; T_6: Hydro-alcoholic extract of box elder 30%; T_7: Aqueous extract of afghanistan pine; T_8: Hydro-alcoholic extract of afghanistan pine 15%; T_9: Hydro-alcoholic extract of afghanistan pine 30%; T_{10}: Aqueous extract of arizona cypress; T_{11}: Hydro-alcoholic extract of arizona cypress 15%; T_{12}: Hydro-alcoholic extract of arizona cypress 30%. As it is shown T_2, T_3, T_5, T_6, T_8, T_9, T_{11} and T_{12} were completely inhibited seed germination of rye-grass

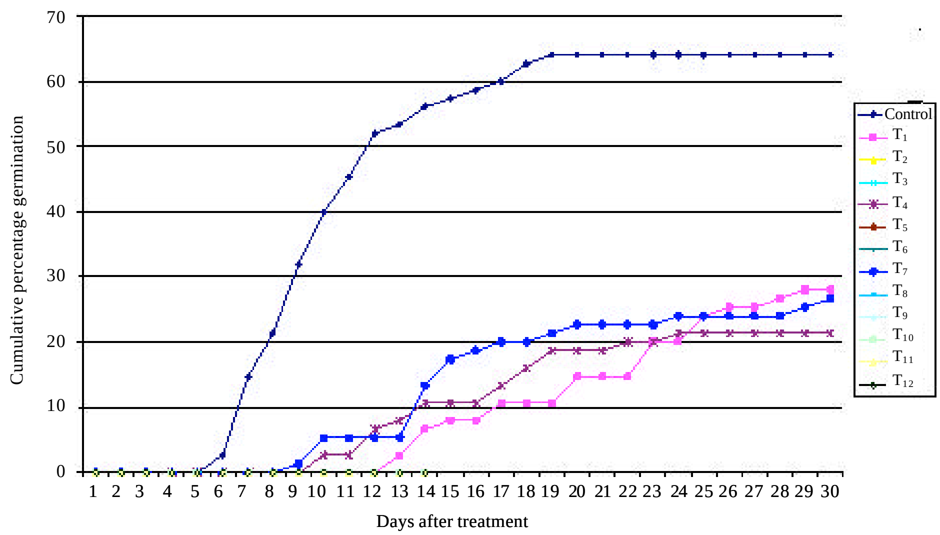

Fig. 2: Effect of different extracts on cumulative percentage germination of *Festuca arundinaceae*. Treatments are as follow: T_1: Aqueous extract of black locust; T_2: Hydro-alcoholic extract of black locust 15%; T_3: Hydro-alcoholic extract of black locust 30%; T_4: Aqueous extract of box elder; T_5: Hydro-alcoholic extract of box elder 15%; T_6: Hydro-alcoholic extract of box elder 30%; T_7: Aqueous extract of afghanistan pine; T_8: Hydro-alcoholic extract of afghanistan pine 15%; T_9: Hydro-alcoholic extract of afghanistan pine 30%; T_{10}: Aqueous extract of arizona cypress; T_{11}: Hydro-alcoholic extract of arizona cypress 15%; T_{12}: Hydro-alcoholic extract of arizona cypress 30%. As it is shown T_2, T_3, T_5, T_6, T_8, T_9, T_{10}, T_{11} and T_{12} were completely inhibited seed germination of tall fescue

alfalfa (*Medicago sativa*), lettuce (*Lactuca sativa*), ryegrass (*Lolium multiflorum*) and *Digitaria sanguinalis*. Increasing the extract concentration increased inhibitory effect. A main inhibitory substance was isolated by spectral data as 9α,13β-epidioxyabeit-8(14)en-18-oic acid. The similar results were previously also observed by Lill *et al*. (1979), who in their results stated that vapour from incubated *Pinus radiata* D. Don litter caused a reduction in the germination of *Lolium perenne* seeds.

The ability of black locust leaf extract to suppress germination of grasses in this experiment agrees with the results of some studies. Nasir *et al*. (2005) stated that the growth of tested species (Chinese cabbage, barnyard grass, white clover and lettuce) was reduced when grown in soil mixed with the leaves of R. pseudo-acacia at various concentrations. Aqueous leaf extracts, exhibited a significant inhibitation of radicle growth. Robinetin, found in a large amount of *R. pseudo-acacia* leaves, caused 50% suppression of the growth of lettuce. Further, Fujii *et al*. (2004) observed that places where these trees dominate others plants and the vegetation beneath the tree is poor.

These results that are in agreement with our finding suggests that leachate from pine needles and black locust leaves may have growth inhibitory substances and possess allelopathic potential whereby could have suppressed germination and growth of competing species.

Based on the literature and observation during this laboratory bioassay on allelopathic effects of different leaf extracts from some woody plants, seems that these trees had potent inhibitory activity on seed germination Rye-grass and Tall Fescue and this inhibition in germination might be due to allelopathic effect. This activity differed depending on the genus and extract type. Results of this study also suggest continual influxes of studied tress leaf leachate into soil during rainfall could have been capable of influencing vegetative composition of landscapes once dominated by them. So, it is recommend to avoid planting these trees and grasses together in landscape, select trees and plants that are allelopathically compatible and use shade-tolerant ground covers, in place of turfgrass around trees. In areas where the lawn is the primary design feature, for reducing allelopathic effects, select woody plants that do the least damage to grass growth and maintenance and regularly clean up all fallen leaves and fruit from trees with allelopathic effects (Appleton *et al*., 2000). Thus, a clear need for an in-depth study of the management of the litter layer beneath tree plantations is necessary. However this experiment was a priming test and further research should focus on the confirmation of the allelopathic interaction in nature and also clarify the effects of other parts of these trees on ground cover.

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