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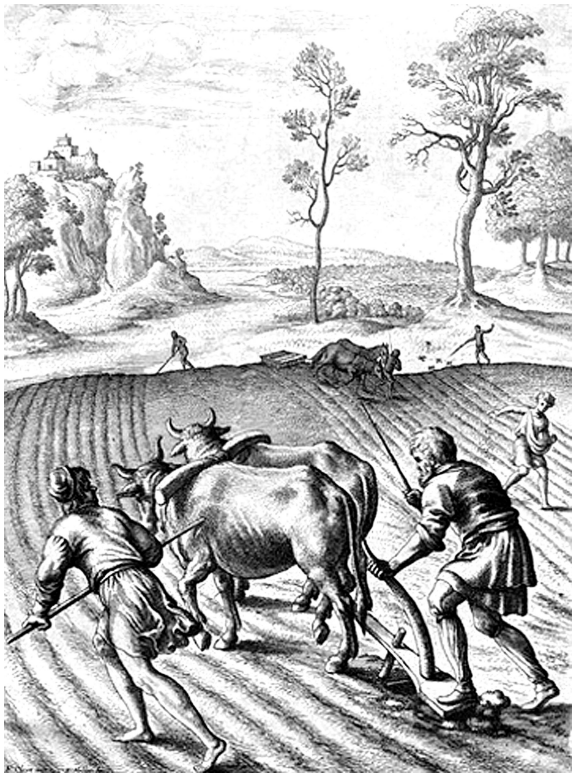

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The Use of Allelochemicals to Delay Germination of *Astragalus cycluphyllus* Seeds

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Abstract: The effect of different concentrations of allelochemicals including Ephedrin, Vanillin, abscisic acid (ABA), Eucalyptus comadulensis and Juglans ragia leaf and Onobrychis sativa seed extracts were tested on percentage germination, germination start and seedlings growth of *Astragalus cycluphgllus*. All considered compounds delayed germination start with respect to the control but among these allechemicals different concentrations of ABA and 33.3 mM of Vanillin delayed germination for a longer period than other allelopathic compounds. Ephedrin, Vanllin and *Eucalyptus camadulensis* leaf extract reduced percentage of germination. In addition, in Ephedrine, *Juglans regia* compounds seedlings had abnormal growth and twisted form. Therefore, only ABA had no negative effect on percentage of germination and seedling growth.

Key words: Allelochemicals, allelopathy, dormancy, germination delay

INTRODUCTION

Chemicals released from plants and imposing allelopathic influences are called allelochemicals or allelochemics[1]. Most allelochemicals are classified as secondary metabolites of plant, which are generally considered those compounds, which do not play a role in primary metabolic processes essential for a plant's survival and are produced by primary metabolic pathways[2,3]. Varieties of allelochemicals have been identified including the phenolic acids, cumarins, terpenoids, flavonoids, alkaloids, glycosides and glucosinolates[4,5]. Chemicals with allelopathic potential are present in almost all plants and in many tissues such as leaves, stems, flowers, fruits, seeds and roots[6] that these chemicals under specific conditions are released in to the environment[7] and can positively or negatively affect the growth and development of vegetation[8-10]. Nowadays, allelochemicals are important in agricultural practices such as weed control[11,12]. Inter cropping, nutrient recycling and low-external input farming[13]. Also, findings indicate that present researches are oriented to the role and mode of action of allellochemicals in order to use their activity in the steps that are involved in the synthesis and control in plant hormone levels[14]. Legumes such as *Astragalus cycluphyllus* are usually cultivated in the spring but there are some problems about the time of cultivation in cold and semi-cold regions. First, at the beginning of the spring pastures are not ready for the entry of workers and machinery to cultivate seeds. Although in the middle of spring, there is insufficient rainfall for seedling stabilization. Second, seedlings cannot stabilize in cold weather. Therefore, seedlings must be done when there is enough moisture and a suitable temperature. Reports indicate that some allelochemicals delay germination[15]. Therefore, in the present study considered the effect some allelopathic compounds on germination start and seedling growth and allelochemicals that had no negative effects on seedling growth or percentage of germination to delay in germination start of seeds. The results of this study can be used for coating of *Astragalus cycluphyllus* seeds with suitable concentration of selected allelochemicals by seed pelleting method in order to cultivate them in the fall but their germination will be delayed until the temperature became suitable.

MATERIALS AND METHODS

In this research the effect of some allelopathic compounds including Ephedrine, Vanillin, ABA, extracts of 40% weight-volume extracts of *Eucalyptus camadulensis* leaf and *Onobrychis sativa* seed (40 g of powdered Eucalyptus leaf and Onobrychis seed in 100 mL of distilled water) and 30% weight-volume extract of *Juglans regia* leaf (30 g of powdered walnut leaf in 100 mL of distilled water) were tested on germination indicators (percentage of germination, germination start, coefficient of velocity, coefficient of allometry and shoot and root dry weight ratio) of *Astragalus cycluphyllus* seeds. In the first stage of test by doing preliminary experiments, suitable concentrations (concentrations which delay

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germination and had no effect on percentage germination) for each allelochemical were selected as below. Concentrations of 33, 23 and 20 mM for Ephedrine and Vanillin, 0.4, 0.7 and 1 mM for ABA and 80 and 100% *Eucalyptus camadulensis* and *Juglans regia* leaf extracts and *Onobryichis sativa* seed extract. For each compound four replicates of 20 seeds were placed in 9 cm diameter petridishes lined with one layer of whatman filter paper No.1 wetted with 3 mL of distilled water (control) or solutions of the given allelochemical at the start. An additional milliliter of each solution was added every 24 h to third day that thereafter added distilled water instead of allelopatic compounds every 24 h because of continuous addition of allelophthic compounds inhibit germination completely. Petridishes then were placed in a germination chamber with germination conditions of 20°C, darkness and relative humidity of 90%. Germinator was evaluated every two days. A seed was considered germinated when the radical protruded ≥ 2 mm[16].

Indicators of Percentage of Germination (PG), Germination Start (GS), Coefficient of Velocity (CV) and Coefficient of Allometry (CA) were calculated according to the following formulas:

Percentage germination = n/N

Where, n is the number of seed germinated and N is the number of sowed seeds.

Germination start = long of time between seeds sowing and beginning of germination is consider as germination start.

Coefficient of velocity = 100 ($\sum$Ni/$\sum$Ni Ti)

Where, N is the number of seeds germinated on day I and T is the number of days from sowing.

Coefficient of allometry (CA) = Ls/Lr

Where, Ls is shoot length and Lr is root length.

Dry weight ratio = (DWs / DWr)

Where, DWs is dry weight of shoot and DWr is dry weight of root. When the seedlings produced two leaves, seedlings were harvested and separated in root and shoot and were kept in 80°C for 24 h and then weighted.

Statistical analyses of data: Data were analyzed using SAS method and the design was a Randomized Complete Block. In addition, Duncan's Multiple Range Test was used to determine significant difference among mean value at the 0.0l probability levels.

RESULTS AND DISCUSSION

Results of effect of different concentrations of allelochmicals on PG indicated that allelopathic compounds of Ephedrine, Vanillin and *Eucalyptus camadulensis* leaf extract reduced PG as compared with control (not shown). Findings show that several allelopathic compounds are structurally similar to plant hormones[14]. In addition, some mechanisms of action of allelochemicals seem to resemble those of synthesis plant hormones[15]. Thus, these compounds probably affect inducible hormones of germination such as gibberellin[8,15] or activity of specific enzymes such as amylases and proteinases which are necessary for seed germination[8]. Therefore, PG decreasing in treated seeds with these allechemicals is expected. The effects of allelopathic compounds on the activity of hormones are considered by experiments done on phenolic growth inhibitors from *Salix rubra* and apple tree which prove to suppress the activity of IAA and gibberellin (GA)[8]. In addition, other considered allelochemical did not influence on PG of the seeds. All compounds delayed GS with respect to the control but among considered compounds ABA was most effective and delayed GS for a longer time than the control (Table 1). Experiments done on several plant species indicate that ABA plays a key role in induction and maintenance of dormancy[17,18]. Also, among considered different concentrations of allechemicals, GS in treated seeds with concentration of 0.4, 0.7 and 1 mM of ABA 33.3 mM of Vanillin were delayed for a longer period than

Table 1: Effect of different allelochemicals on germination start of *Astragalus cycluphyllus* seeds. Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences

Treatments	Materials concentration according to mM			
Vanillin	Control	20	25	33.3
	3^{i}	7^{efg}	10^{d}	14^{c}
Ephedrin	Control	20	25	33.3
	3^{i}	5^{ghi}	5^{ghi}	5^{ghi}
Abscisic acid	Control	0.4	0.7	1
	3^{i}	26.5^{b}	27.7^{b}	29^{a}
Eucalyptus	Material concentration according to % weight-volume			
camadulensis	Control	80%		100%
leaf extract	3^{i}	8^{def}		8^{def}
Juglans regia	Control	80%		100%
leaf extract	3^{i}	9^{de}		4^{de}
Onobrychis sativa	Control	80%		100%
seed extract	3^{i}	6^{fgh}		7^{efg}

Table 2: Effect of different allelochemicals on coefficient of velocity of *Astragalus cycluphyllus* seeds. Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences

Treatments	Materials concentration according to mM			
Vanillin	Control	20	25	33.3
	0.21^{ab}	0.112^{e}	0.086^{f}	0.088^{f}
Ephedrin	Control	20	25	33.3
	0.23^{a}	0.185^{c}	0.161^{c}	0.177^{c}
Abscisic acid	Control	0.4	0.7	1
	0.22^{a}	0.03^{g}	0.028^{g}	0.027^{g}
Eucalyptus	Material concentration according to % weight-volume			
camadulensis	Control	80%		100%
leaf extract	0.23^{a}	0106^{e}		0.09^{f}
Juglans regia	Control	80%		100%
leaf extract	0.21^{ab}	0.098^{f}		0.091^{f}
Onobrychis sativa	Control	80%		100%
leaf extract	0.21^{ab}	0.131^{d}		0.116^{e}

Table 3: Effect of different allelochemicals on coefficient of allometry of *Astragalus cycluphyllus* seedlings. Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences

Treatments	Materials concentration according to mM			
Vanillin	Control	20	25	33.3
	1.4^{abcde}	1.5^{abcde}	1.65^{abc}	1.26^{cdef}
Ephedrin	Control	20	25	33.3
	1.4^{abcde}	1.75^{a}	1.75^{a}	1.48^{abcd}
Abscisic acid	Control	0.4	0.7	1
	1.5^{abcde}	1.48^{abcd}	1.68^{ab}	1.64^{abc}
Eucalyptus	Material concentration according to % weight-volume			
camadulensis	Control	80%		100%
leaf extract	1.5^{abcde}	1.08^{ef}		1.08^{ef}
Juglans regia	Control	80%		100%
leaf extract	1.4^{abcde}	1.06^{ef}		1.15^{def}
Onobrychis sativa	Control	80%		100%
seed extract	1.4^{abcde}	1.35^{abcde}		1.34^{bcde}

Table 4: Effect of different allelochemicals on shoot to root dry weight ratio of *Astragalus cycluphyllus* seedlings. Average comparison performed using of Duncan's test at the 0.01 level. Different letters indicate significant differences

Treatments	Materials concentration according to mM			
Vanillin	Control	20	25	33.3
	4.1^{bcd}	4.9^{abc}	4.9^{abc}	4.8^{abc}
Ephedrin	Control	20	25	33.3
	4.1^{bcd}	4.05^{cd}	4.05^{cd}	3.9^{cd}
Abscisic acid	Control	0.4	0.7	1
	3.6^{def}	3.1^{ef}	4.1^{bcd}	4.3^{bcd}
Eucalyptus	Material concentration according to % weight-volume			
camadulensis	Control	80%		100%
leaf extract	3.6^{def}	3.4^{def}		4.07^{cd}
Juglans regia	Control	80%		100%
leaf extract	3.9^{d}	3.49^{def}		3.03^{f}
Onobrychis sativa	Control	80%		100%
leaf extract	4.1^{bcd}	4.1^{cd}		4.5^{bcd}

other considered compounds concentrations. Evidences show that exogenous ABA controls germination by limiting water uptake to embryos[19] and probably affects cell wall extensibility[19] or membrane rigidity by biophysical interaction with phospholipid[20]. Also, reports show that transition from dormant to the non-dormant state is marked by changes in the composition of membrane-associated proteins[21,22]. Thus, changes in dormancy may be related to changes at the level of membrane proteins and ABA possibly induces expression of specific genes involved in the blacking of embryo germination[18]. Some allelochemicals such as ferulic acid activate the synthesis of ABA[14] and the antiauxin and antigibberellin activity increased by some terpenes[15]. Also, allelochemicals act upon pathways that are involved in the synthesis and control of plant hormone levels. These could represent a important factor to regulate many metabolic processes that govern plant growth[14]. All allelochemicals decreased CV significantly in respect with the control but treated seeds with ABA had the least CV (Table 2). Generally, CV decreases as less seed germinate and with longer germination time[23]. Thus, ABA delayed GS for a longer time than other allelopathic compounds. Results of the effect of allelochemicals on CA (Table 3) and shoot to root dry weight ratio of *Astragalus cycluphyllus* seedlings (Table 4) show that none of allelochemics had negative effect on seedlings growth but treated seedlings with Ephedrin and *Juglans regia* leaf extract had abnormal and twisted form. Therefore, the effect of allelopathic compounds on different indicators of germination indicated that among considered compounds treated seeds with ABA delayed GS for longer time than other tested compounds. Also, this compound had no negative effect on seedlings growth and development and PG. Thus, based on obtained results from this research is proposed that in order to delay germination of *Astragalus cycluphyllus* seeds in cold and semi-cold regions, seeds can be coated (by one of the seed coating methods) with materials containing ABA. Reports also show that leaves of *Fagus silvatica* and flowers of sugar beet have much ABA[8]. Therefore, it is suggested that the extract of these ABA containing plants can be used for seed coating as a seed germination delayer.

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