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The Effect of Some Allelochemicals on Seed Germination of *Coronilla varia* L. Seeds

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Abstract: In the allelopathy process the released compounds from different parts of a plant can positively or negatively affect growth vegetation and seed germination. Because some problems exist in cold and semi-cold regions about cultivation time of legumes, effect of some allelopathic compounds (Ephedrine, Vanillin, Caffeine, abscisic acid (ABA), *Eucalyptus camadulensis* and *Juglans regia* leaf and *Onobrychis sativa* seed extracts) were considered on germination indicators (Percentage of germination, germination start, Coefficient of velocity, Coefficient of allometry and Dry weight ratio) with the intention of delaying the germination start, to beginning suitable time for purposes of temperature and moisture. Among the considered compounds ABA, leaf extracts of *Eucalyptus camadulensis* and *Juglans regia* (especially ABA) delayed germination for a longer period than other allelopathic compounds. In addition, in Ephedrine, *Juglans regia* compounds, seedlings had an abnormal growth and a twisted form. Thus, only ABA had no negative effect on the percentage of germination and seedling growth and caused delay of germination start for a longer time.

Key words: Allelochemicals · allelopathy · *Coronilla varia* L. · dormancy · germination delay

INTRODUCTION

Chemicals with allelopathic potential exist in almost all plants and most tissues [1] and can positively or negatively affect growth vegetation [2, 3]. Most of the allelochemicals are classified as secondary metabolites in plants, are generally considered those compounds which do not play any role in primary metabolic processes essential for plant's survival [4, 5]. Secondary metabolites can be arranged from pigments to toxic gases and have no internal effect on the plant and some times in the case of pigment, it has a useful effect on attracting pollinators [6]. Some allelochemics are intermediates for lignification and can also activate plant defence after exposure to pathogens. Therefore, they have a structural or physiological role within plants [7]. The recognized importance of allelopathy in agriculture has increased with the main objectives of using allelopathy in biological control of weeds and crop productivity [3, 8-10]. Reports show that Wheat (*Triticum aestivum*) allelopathy has the potential for the management of weeds, pests and diseases [11]. Allelopathy also has been used for intercropping, nutrient recycling and low external input farming practice [12] and affect nutrient uptake [1]. Allelopathy may induce genetic changes within associated plant populations [13] and change the pH of soil [6]. Allelopathic compounds influence physiological processes such as cellular expansion, cell wall construction, phytohormonal balance, activity of specific enzymes (e.g. indolacetic acid oxidase), pollens, spores and seeds germination, mineral uptake, stomatal movement, pigment synthesis, photosynthesis, respiration, protein synthesis, leghemoglobin biosynthesis, N_2 fixation, plant water relations, DNA and RNA modification [3, 14] and activation of cellular antioxidative [15]. Legumes are usually cultivated in spring of cold and semi-cold regions. There are some problems about the cultivation time of Legumes. First, at the beginning of spring pastures are not ready for entry of workers and machinery to cultivate the seeds. Also in the middle of spring, the rainfall is insufficient for stabilization of seedling. Second, seedlings cannot be stabilized in cold weather. Therefore, seedlings must be done in a suitable moisture and temperature. Reports indicate that some allelochemicals delay germination [6]. Therefore, in the present study, the effects of some allelopathic compounds on germination start and seedling growth were considered to choose the allelochemicals that have no negative effect on seedling growth or the percentage of germination in order to delay the germination start of seeds. The results of this study can be used for coating *Coronilla varia* seeds with a suitable concentration of selected allelochemicals by the seed pelleting method in order to cultivate them in the fall but their germination will be delayed until the temperature becomes suitable.

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MATERIALS AND METHODS

Allelochemicals: In order to determine the suitable concentration of some allelochemicals (concentrations which delay germination and have no effect on percentage of germination) the effect of some allelopathic compounds such as Caffeine, 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 examined on the germination indicators (percentage of germination, germination start, coefficient of velocity, coefficient of allometry and shoot and root dry weight ratio) of *Coronilla varia* seeds. The experiment was done in two stages. In the first stage, suitable concentrations for each allelochemical were selected by doing preliminary experiments, as below. Concentrations of 40, 43.4, 47.6 and 55 mM for Caffeine, 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. Also because *Coronilla varia* seeds have a hard coat, therefore seeds were scarified by sandpaper. For each compound four replicates of 20 seeds were placed in a 9 cm diameter Petri dish lined with one layer of Whatman filter paper No.1 wetted with 3 ml of distilled water (control) or a solutions of the allelochemicals mentioned. An additional ml of each solution was added every 24 h till the third day. Thereafter distilled water was added instead of allelopathic compounds every 24 hours because continuous adding of allelophthic compounds will completely inhibit germination. Then the petri dishes were placed in a germination chamber with germination conditions of 20°C, darkness and relative humidity of 90%. Germination was evaluated every two days. A seed is considered germinated when the radical is protruded = 2 mm [16].

Germination and growth indicators: 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 [17]:

1) Percentage of germination = 100(n/N)
   Where n is the number of seed germinated and N is the number of sowed seeds.
2) Germination start = The long time between seed sowing and beginning of germination.
3) Coefficient of velocity = 100 (ÓNi / ÓNi Ti)
   Where N is the number of seeds germinated on day i and T is the number of days from sowing.
4) Coefficient of allometry = Ls/Lr
   Where Ls is shoot length and Lr is root length.
5) 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 analysis: Data were analysed using SAS method and the design was a randomised-complete-block. In addition, Duncan's multiple range tests were used to determine significant difference among mean value at the 0.0l probability levels.

RESULTS AND DISCUSSION

In the present study, we have shown that allelopathic compounds of Caffeine and Ephedrine reduced PG as compared with the control (Table 1). Findings show that several allelopathic compounds are structurally similar to plant hormones [18]. In addition, some mechanisms of action of allelochemicals seem to resemble those of synthesis plant hormones [6]. Thus, these compounds probably affect inducible hormones of germination such as gibberellin [3, 6] or activity of specific enzymes such as amylases and proteinases, which are necessary for seed germination [3]. Therefore, decrease of PG is expected in the treated seeds with these allelochemical. 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) (Kefeli and Turetskaya, 1967 cited in 3]. In addition, other considered allelochemical did not influence PG of the seeds. The effects of allelochemics on GS of *Coronilla varia* seeds are summarized in Table 2. Among considered compounds ABA, *Eucalyptus camadulensis* and *Juglans regia* leaf extracts, also concentrations of 33.3mM of Ephedrine and 100% *Onobrychis sativa* seed extract have significant effects on GS. Also in treated seeds with ABA, GS was delayed for a longer period than other allelochemicals. Experiments done on several plant species indicate that ABA plays a key role in induction and maintenance of dormancy [19, 20]. Evidence shows

Table 1: Effect of different allelochemicals on percentage of germination of *Coronilla varia* 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				
Caffeine	Control	40	43.4	47.6	55
	97.5^{ab}	85^{e-g}	90^{a-d}	85^{e-g}	72.5^{g}
Vanillin	Control	20	25	33.3	
	97.5^{ab}	100^{a}	97.5^{ab}	95^{a-c}	
Ephedrine	Control	20	25	33.3	
	97.5^{ab}	95^{a-c}	85^{c-f}	77.5^{fg}	
Abscisic acid	Control	0.4	0.7	1	
	97.5^{ab}	92.5^{a-d}	90^{a-d}	90^{a-d}	
Eucalyptus camadulensis leaf extract	Material concentration according to % weight-volume				
	Control	80%	100%		
	97.5^{ab}	95^{a-c}	90^{a-d}		
Juglans regia leaf extract	Control	80%	100%		
	97.5^{ab}	97.5^{ab}	100^{a}		
Onobrychis sativa seed extract	Control	80%	100%		
	100^{a}	92.5^{a-d}	92^{a-d}		

Table 2: Effect of different allelochemicals on germination start of *Coronilla varia* 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				
Caffeine	Control	40	43.4	47.6	55
	3^{fg}	5^{e-g}	5^{e-g}	5^{e-g}	5^{e-g}
Vanillin	Control	20	25	33.3	
	3^{fg}	5^{e-g}	7^{c-g}	8^{c-f}	
Ephedrine	Control	20	25	33.3	
	3^{fg}	5^{e-g}	7^{c-g}	10^{b-e}	
Abscisic acid	Control	0.4	0.7	1	
	3^{fg}	27^{a}	27.5^{a}	34^{a}	
Eucalyptus camadulensis leaf extract	Material concentration according to % weight-volume				
	Control	80%	100%		
	3^{fg}	11.5^{bc}	13^{b}		
Juglans regia leaf extract	Control	80%	100%		
	3^{fg}	11^{bc}	13^{b}		
Onobrychis sativa seed extract	Control	80%	100%		
	3^{fg}	6^{d-g}	10.5^{b-d}		

that exogenous ABA controls germination by limiting water uptake to embryos [21] and probably affects cell wall extensibility [22] or membrane rigidity by biophysical interaction with phospholipid [23]. Also, reports show that transition from dormant to non-dormant state is marked by changes in the composition of membrane-associated proteins [24, 25]. 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 blocking of embryo germination [24]. Some allelochemicals such as ferulic acid activate the synthesis of ABA [18] and the antiauxin and antigibberellin activity increased by some terpenes [6]. Also, allelochemicals act upon pathways that are involved in the synthesis and control of plant hormone levels. These could represent an important factor to regulate many metabolic processes that govern plant growth [18]. Table 3 shows the effect of the allelochemicals on the CV of *Coronilla varia*. All allelochemicals decreased CV significantly in respect with the control, but seeds treated with ABA had the least CV. Generally, CV decreases as less seed germinate and with a longer germination time [17]. Thus, ABA delayed GS for a longer time than other allelopathic compounds. Results of the effect of allelochemicals on CA (Table 4) and shoot to root dry weight ratio of *Coronilla varia* seedlings

Table 3: Effect of different allelochemicals on coefficient of velocity of *Coronilla varia* 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				
Caffeine	Control	40	43.4	47.6	55
	0.28^{a}	0.14^{c-f}	0.12^{c-h}	0.11^{d-h}	0.10^{e-h}
Vanillin	Control	20	25	33.3	
	0.28^{a}	0.16^{b-e}	0.12^{c-h}	0.098^{e-h}	
Ephedrine	Control	20	25	33.3	
	0.28^{a}	0.14^{c-f}	0.11^{d-h}	0.085^{e-h}	
Abscisic acid	Control	0.4	0.7	1	
	0.29^{a}	0.035^{f-h}	0.033^{gh}	0.025^{h}	
Eucalyptus camadulensis leaf extract	Material concentration according to % weight-volume				
	Control	80%	100%		
	0.28^{a}	0.068^{e-h}	0.060^{e-h}		
Juglans regia leaf extract	Control	80%	100%		
	0.28^{a}	0.072^{e-h}	0.073^{e-h}		
Onobrychis sativa leaf extract	Control	80%	100%		
	0.28^{a}	0.14^{c-f}	0.085^{e-h}		

Table 4: Effect of different allelochemicals on coefficient of allometry of *Coronilla varia* 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				
Caffeine	Control	40	43.4	47.6	55
	2.19^{c}	3.27^{a}	3.2^{a}	3.12^{ab}	3.15^{ab}
Vanillin	Control	20	25	33.3	
	1.96^{cd}	1.92^{cd}	1.92^{cd}	1.72^{c-f}	
Ephedrine	Control	20	25	33.3	
	1.96^{cd}	1.62^{d-f}	1.61^{d-f}	1.91^{cd}	
Abscisic acid	Control	0.4	0.7	1	
	1.93^{cd}	1.69^{d-f}	1.61^{d-f}	1.6^{d-f}	
Eucalyptus camadulensis leaf extract	Material concentration according to % weight-volume				
	Control	80%	100%		
	1.92^{cd}	1.73^{d}	1.83^{c-e}		
Juglans regia leaf extract	Control	80%	100%		
	1.93^{cd}	1.72^{de}	1.79^{c-e}		
Onobrychis sativa seed extract	Control	80%	100%		
	1.96^{cd}	1.93^{cd}	1.62^{d-f}		

(not shown) indicate that none of allelochemics (with the exception of Caffeine) had negative effect on seedlings growth but treated seedlings with Ephedrine and *Juglans regia* leaf extract had abnormal and twisted form (not shown). Therefore, results of the present research indicate that among the considered compounds, ABA delayed GS for a 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, it is proposed that in order to delay germination of *Coronilla varia* seeds in cold and semi-cold regions, seeds can be coated (by one of the seed coating methods) with materials containing ABA. Also reports show that leaves of *Fagus silvatica* and flowers of sugar beet have much ABA [3]. 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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