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Assessment of decreasing of allelopathic effect of *Juglans regia* on germination properties of *Melilotus officinalis* under Influence of chemical stimulators

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Abstract

This test was conducted on laboratory condition to investigate the allelopathic effect of *Juglans regia* on germination and early seedling growth of *Melilotus officinalis*. Pretreatment with gibberellic acid and salicylic acid also was used to decrease the adverse effect of *allelopathic* components. Three levels of gibberellic acid (125, 250 and 500 ppm) and salicylic acid (100, 200 and 300 mg/L) and five extract levels of *Juglans regia* (0, 25, 50, 75 and 100 %) was used in the test. This experiment was carried out as factorial experiment based on a randomized completely design with four replications. The results of ANOVA and compare mean test showed that *Juglans regia* extract had inhibitive effect on germination and early seedling growth of *Melilotus officinalis*. Early seedling growth of *Melilotus officinalis* increased by pretreatment of seeds in chemical stimulators so that the highest effect was observed in gibberellic acid. Interaction effects of allelopathic and pretreatment with chemical stimulators were significance on germination percentage and speed, root, shoot and plant length and seed vigour index.

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Introduction

Successful plant establishment, the most important step in determining the competitive ability and regeneration of range and crop plants, depends on germination potency and plant growth. Allelopathic compounds restrict plant growth through negative interactions with important physiological processes such as changing cell wall structure; prevent of cell division and activity of some enzymes. These compounds can also affect the equilibrium of plant hormones, absorption of nutrient elements, displacement of stomata, photosynthesis, respiration, protein synthesis, pigment, and changes in DNA and RNA structures (Glass & Bot, 1974). Allelochemicals have inhibitory or stimulating effects on the growth, health, behavior, and biotic population of other organisms (plants, insects, microbes, etc.) (Zhang *et al.*, 2003).

Priming is used to improve germination, reduce germination time and embryo emergency, and improve establishment and performance of species (Ghobadi *et al.*, 2012, Saberi *et al.*, 2011, saberi & tarnian, 2012). Priming also applies to increase seed vigority and reduce losses from late plantation. Many researchers have reported that priming can increase germination percentage and emergence of weakened or damaged seeds (Horii *et al.*, 2007).

One of the plant hormones that can reduce the negative effect of allelochemicals is salicylic acid. It is an endogenous growth regulator of phenolic nature which participates in the regulation of physiological processes in plant (Raskin, 1992). These include effect on ion uptake, membrance permeability, etc. (Camberato & Mccarty, 1999). In addition, salicylic acid intract with other signaling pathways including those regulated by jasmonic acid and ethylene (Ding & Wang, 2003). Salicylic acid induces an increase in the resistance of seedling to osmotic stress (Borsani *et al.*, 2001), low or high temperature by activation of glutathione reductase and guaiacol peroxidase (Kang *et al.*, 2002), and toxic action of heavy (Mazen, 2004) by activation of systemic acquired resistance. (Ghobadi *et al.*, 2012) Reported that pretreatment the seeds of *Agropyron elongatum* and *Bromus inermis* by chemical stimulators (gibberellic acid, potassium nitrate and salicylic acid) decreased the allelopathic effect of *Thymus kotschyanus*. It has been pointed to salicylic acid as intermediate for reacting to abiotic stress (Bor & Ozdemir, 2003).

Juglans regia is one of the most important plants used to prevent soil erosion and to recover the plant cover in studied area. This plant also used in farmland as medical plant. In studied area which consists of 6000 hec, many plants (range species, cultivatable and medical plant) were cultivated based on different goals. The objective of this study was to evaluate the effectiveness of seed priming in improving seed germination and seedling vigour of *Melilotus officinalis*, in response to allelopathic effects of *Juglans regia* under laboratory conditions.

Materials and methods

This research was carried to determine the effect of chemical stimulators used to reduction of allelopathic effect of *Juglans regia* on germination and primary growth of *Melilotus officinalis*. To meet this aim, at first, aerial and underground parts of *Juglans regia* were collected from Niasar, Esfahan, Iran. After air drying at room temperature, 5 g of powder was picked and mixed in 100 mL water, placed on a shaker for 24 h then centrifuged at 3000g for 15 min. The obtained mixture was filtered using Whatman 1 filter paper. Concentrations of 25, 50, 75 and 100% were prepared using centrifuged solution. Seeds of *Melilotus officinalis* (collected from Niasar farm and rangeland) were disinfected by using 5% solution of sodium hypochlorite before starting of test and were washed by using distilled water several times. Then seeds were pretreated using salicylic acid 100, 200 and 300 mg for 10 hours and using gibberellic acid 125, 250 and 500 ppm for 24 hours at 25ċ temperature and distilled water were used as control treatment simultaneously. All seeds were washed with distilled water after soaking period and then were placed into petri dishes with dimensions of 9cm on a filter paper (Watman 1) after being dried in order to test different stress conditions with various concentration of

allelopathic extract related *Juglans regia*. Petri dishes were steriled for 48 hours in the oven at 20ċ before placing seeds. Germination test was performed using factorial test (5×7) in completely randomized design with 4 replications (25 seeds per Petri dishes) in different concentration of extract related to *Juglans regia* (0, 5, 25, 50 and 75 percentage) and 25 ċ in the germinator. Germinated seeds that had length more than 2mm were counted each day over a period of 10 days (Kaya *et al.*,2006) and germination percentage, germination speed, root length shoot length, plant length and vigour index of seed were measured.

Germination percentage (Camberato & Mccarty, 1999) and germination speed were measured based on equations at follow:

(1) Germination percentage

$$GP = \frac{\sum G}{N} \times 100$$

GP: germination percentage, G: number of germinated seeds, N: number of seeds

(2) germination speed

$$GR = \sum_{i=1}^{n} \frac{S_i}{Di}$$

Si: number of germinated seed at each counting, Di: number of day until n counting, n: numbers of counting

(3) plant length = root length + shoot length

(4) vigourindex $V_{i=} \frac{\%Gr \times MSH}{100}$

Vi: vigour index, MSH: mean of plant length (root length + shoot length) per mm, Gr: Germination percentage.

The obtained data was analyzed using analysis of variance (ANOVA). Means were compared at 5% level of significance using Duncan's multiple range tests with statistical software MSTAT-C version 2.00.

Results of variance analysis (table 1) showed that chemical stimulators and various concentrations of *Juglans regia* and interaction effect of them had significant effect on all studding properties of *Melilotus officinalis* species (P<0.01).

Germination percentage and speed

The results showed that allelopathic extract of *Juglans regia* caused decrease in germination percentage of *Melilotus officinalis*. Differences were significance between control treatment and various concentrations of extract. All the chemical stimulators could increase germination percentage of *Melilotus officinalis* seeds comparing to control treatment (fig. 1). Interaction effects of chemical stimulators and various concentrations of *Juglans regia* on germination speed of *Melilotus officinalis* seeds were significance (P<0.01) (Fig. 2). Results showed that germination speed of seeds that were exposure of various concentrations of extracts had significant differences with the control treatment. allelopathic extract caused decrease in germination speed of *Melilotus officinalis*. Using of the chemical stimulators have effect on germination speed so that 250 ppm gibberellic acid has high effect on germination speeds by comparing to control treatment (fig. 2).

Root length

Interaction effect of chemical stimulators and various concentrations of extract of *Juglans regia* were significance on root length. All stimulators improved root length in stress condition with extract of *Juglans regia* so that the highest root length was related to use 100 ppm salicylic acid (fig. 3).

Shoot length

Results also indicated that Interaction effects of chemical stimulators and various concentrations of extract of *Juglans regia* were significance on shoot length. The highest shoot length of *Melilotus officinalis* was related to use of gibberllic acid treatment in stress and non stress conditions. Various concentrations of *Juglans regia* reduced shoot length of it. Chemical stimulators caused an increase in shoot length in stress condition which differences were significance (p<.01) (Fig. 4).

Plant length

Mean comparison test showed that interaction effect of chemical stimulators and various concentrations of *Juglans regia* were significant on plant length so that plant length reduced by increasing concentration of *Juglans regia*. All concentrations of chemical stimulators caused an increase in plant length of *Melilotus officinalis* in stress condition. The highest plant length was related to use of 100 ppm salicylic acid and 250 and 500 ppm gibberellic acid in stress and non stress conditions (fig. 5).

Seed vigour index

Mean comparison showed that interaction effect of chemical stimulators and various concentrations *Juglans regia* were significance on vigour index. Results showed that vigour index reduced by increasing the concentration of *Juglans regia* and it was significance by comparison to the control treatment. Chemical stimulators increased seed vigority as the highest increase was related to 250 ppm gibberellic acid (fig. 6).

Discussion and conclusion

Environmental and nonenvironmental stresses can cause some adverse reactions in plants. One group of environmental stresses consists of allelopathic compounds secreted by some plants that may disrupt the lifecycles and activate some biochemical reactions in other plants. Our research showed that chemical stimulators played a key role in the reduction of stress from allelopathic compounds of *Juglans regia* on seed germination and primary growth of *Melilotus officinalis*. Use of gibberellic acid and Salicylic acid increased germination percentage, root length, shoot length, plant length, and seed vigour index of *Melilotus officinalis* significantly under stress and non-stress conditions. These results are coincide with the results of (saberi *et al.*, 2012) who stated chemical stimulators such as gibberellic and salicylic acid could be used as stimulator for improving germination under allelopathic condition. Plant hormones such as gibberellic acid have an important role in germination process (Ritchie & Gilroy, 1998).

Table 1. Variance analysis of studied traits of *Melilotus officinalis*.

Properties		A	B	A*B	Error
	df	6	4	24	105
Germination percentage	ss	5694.3	18942.9	3577.1	7325
	ms	949	4735.7	149	69.8
	F	13.6**	67.9**	2.1**	-
	df	6	4	24	105
Germination speed	ss	54.5	149.8	32.4	63.4
	ms	9.1	37.5	1.4	0.6
	F	15.1**	62.0**	2.2**	-
	df	6	4	24	105
Root length	ss	11.2	85	12.6	8.1
	ms	1.9	21.3	0.5	0.08
	F	24.1**	275.2**	6.8**	-
	df	6	4	24	105
Shoot length	ss	7	13.7	1.5	4.3
	ms	1.2	3.4	0.06	0.04
	F	28.3**	83.2**	1.5**	-
	df	6	4	24	105
Plant length	ss	13.8	166.4	17.5	9.5
	ms	2.3	41.6	0.7	0.09
	F	25.5**	461**	8.1**	-
	df	6	4	24	105
Seed vigor	ss	97597.8	1472792.7	149826.4	129191.3
	ms	16266.3	368198.2	6242.8	1230.4
	F	13.2**	299.3**	5.1**	-

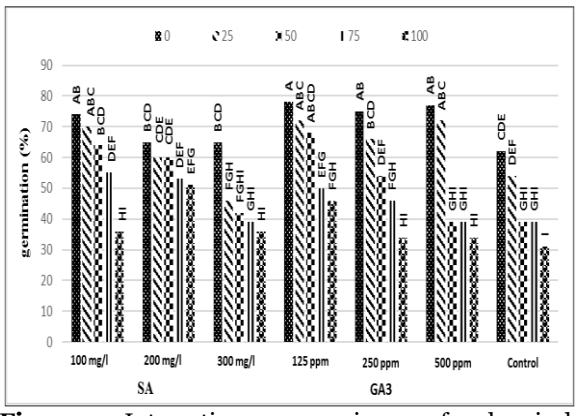

Fig. 1. Interaction comparison of chemical stimulators and various concentrations on germination of *Melilotus officinalis*.

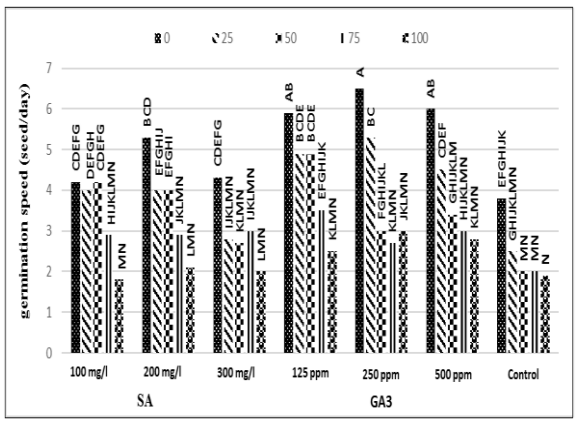

Fig. 2. Interaction comparisons of chemical stimulators and various concentrations on germination speed of *Melilotus officinalis*.

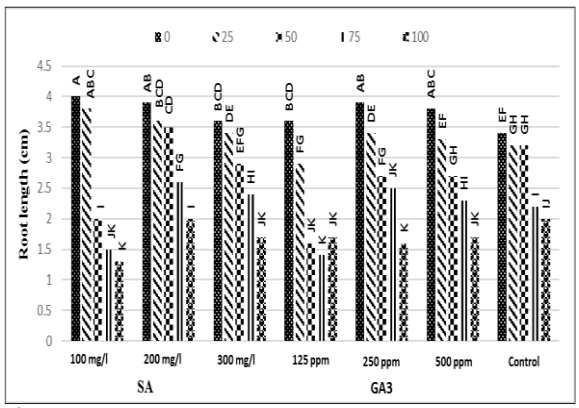

Fig. 3. Interaction comparison of chemical stimulators and various concentrations on root length of *Melilotus officinalis*.

Gibberellic acid produced during germination is used directly in plant growth through hydrolysis of storage foods (Kepczynski, & Groot 1989). It increases enzymatic synthesis such as hydrolytic enzymes. Synthesized enzymes transfer to endosperm and cause digestion of reserve food and provide supply of energy for germination and growth. Delay or stimulate in digestion of reserve food may cause lack of productions of respiration and consequently caused lack of ATP in seeds exposed to allelochemicals. Disorder in respiration results limits in metabolic energy and causes decrease in germination and early seedling growth (Cirac *et al*., 2004). Presoaking seeds with different levels of salycilic acid exposure to extract effect of Obtained results from (Tasgin *et al*., 2003, saberi, & tarnian, 2012) Also verify this hypothesis that salicylic acid is a suitable stimulator for seed germination. Salicylic acid increases germination by neutering of free radicals or active oxygen (Kang & Saltveit, 2002), increasing of antioxidant such as ascorbate (Baalbaki *et al*., 1999), decreasing of ion transfers and accumulation of toxic ions (Krantev, 2008) and increasing of some plant hormones such as auxins and cytokinins (Sharikova *et al*., 2003). In addition to effect of salicylic acid in increasing plant growth in stress condition, this research confirms importance of these phenolic compounds on improvement of initial growth stage when seeds expose to stress condition with *Juglans regia*.

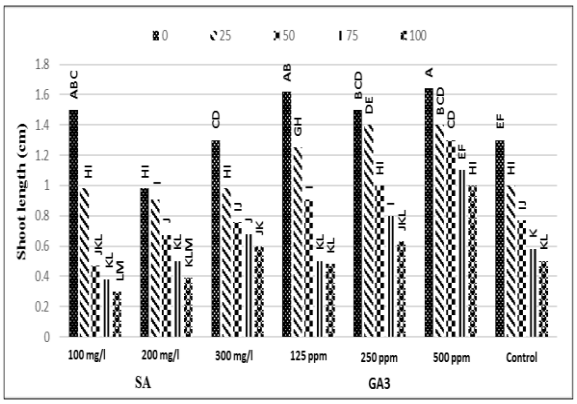

Fig. 4. Interaction comparison of chemical stimulators and various concentrations on shoot length of *Melilotus officinalis*.

Over all, chemical growth stimulators can reduce the inhibitory effects of the allelopathic compounds of *Juglans regia* on the seed germination and seedling growth of *Melilotus officinalis*. Pretreatment with gibberellic acid had more effect than salicylic acid on germination properties.

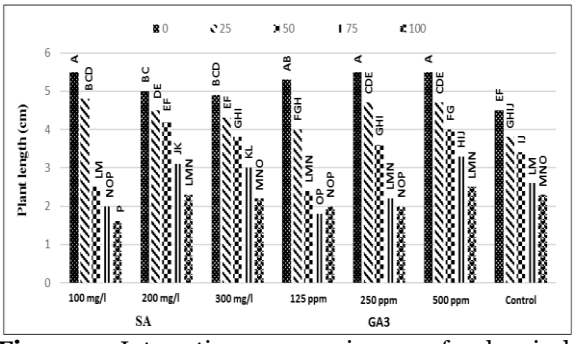

Fig. 5. Interaction comparisons of chemical stimulators and various concentrations on plant length of *Melilotus officinalis*.

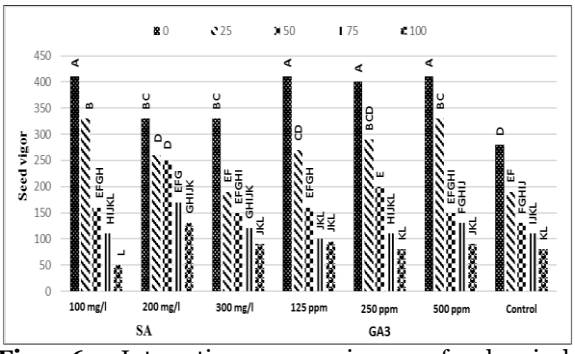

Fig. 6. Interaction comparisons of chemical stimulators and various concentrations on seed vigor of *Melilotus officinalis*.

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