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EFFECT OF SEED PRIMING ON IMPROVEMENT OF GERMINATION OF *VICIA VILLOSA* UNDER ALLELOPATHIC COMPONENTS OF *EUCALYPTUS CAMALDULENSIS*

ABSTRACT

The objective of this study was to evaluate the effectiveness of seed priming in improving seed germination and seedling vigor of *Vicia villosa* under laboratory conditions. Chemical stimulators included: gibberellic acid (125,250 and 500 ppm), salicylic acid (100,200 and 300 mg/lit) and extract of *Eucalyptus camaldulensis* (0, 25, 50, 75 and 100 %). This experiment was carried out as factorial experiment based on a randomized completely design, with four replications. The results showed that *Eucalyptus camaldulensis* extract had inhibitive effect on germination and early seedling growth of *Vicia villosa*. Early seedling growth of *Vicia villosa* increased by pretreatment of seeds in chemical stimulators so that the highest effect was observed in gibberellic acid (250 ppm). The chemical stimulators don't have any effect on germination speed. Interaction effects of allelopathic and pretreatment with chemical stimulators were significance on germination percentage, root, shoot and plant length and seed vigor index.

Key words: allelopathy, germination, chemical stimulators, *Eucalyptus camaldulensis, Vicia villosa*

INTRODUCTION

Germination is one of the most important stages on plant life cycle since this controls establishment of plants and determines the final function of them. Poor establishment of plant is a main reason of decreasing its function in arid and semiarid environment (Afzal, 2005; Harris *et al.,* 2001). In this arias, soil environment often are not suitable for high germination and growth of plants. Biotic and abiotic factors such as lack or extras of water

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and high elements can decrease the germination speed and percentage or inhibit them completely (Ashraf and Foolad, 2005). Improvement of germination can increase establishment of plant spatially in stress condition (He *et al.,* 2002). Allelopathy, as a stress, is defined as direct and indirect effects of allelochemical compound resulted from organism which may have inhibitive or stimulative effects on the same or different organism. Allelopathic components restrict growth of plant through interaction in important physiological process such as change in cell wall structure, infiltration and function of membrane, prevention of cell division and activity of some enzymes. These components also can cause effect on equilibrium of plant hormones, absorption of nutrient elements, displacement of stomata, photosynthesis, respiration, protein synthesis and pigment and change in DNA and RNA structures (Glass, 1974). This phenomenon has direct effect on plants in agricultural ecosystems or indirect effects in biological or non biological process through the same or the other plants (Inderjit, 2001).

Priming is used in germination improvement, reduction of germination time and embryo emergency, and improvement of establishment and performance. Priming also applies in increasing of seed vigority and reduction of 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).

Different physiological and biochemical effects from salicylic acid on plant systems have been observed that include ion absorption, membrane permeability, mitochondrial respiration, effect on stomata, growth rate and photosynthesis rate (Senaranta, 2003). Furthermore, salicylic acid causes production of phenolic matter which acts as blockage in cell wall and then decreases losses of water and also inhibits spread of diseases. Phenols acts as antioxidant in plant and causes to trap free radicals by antioxidant process (Burguieres *et al.,* 2007). It is determined that salicylic acid decreased ion leakage and accumulation of toxic ions significantly in plants (Krantev *et al.,* 2008; Zhou *et al.,* 2009) and caused decrease the effect of environmental stress via increase of hormones such as auxins and cytokinins (Shakirova *et al.,* 2003). Saberi *et al.* (2011) 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 (Naser- alavi *et al.,* 2008; Bor *et al.,* 2003).

Pre-treatment with chemical substance had been known as a simple technique, low cost and risk which suggested improving germination and seedling. The objective of this study was to evaluate the effectiveness of seed priming in improving seed germination and seedling vigor of *Vicia villosa*, in response to allelopathic effects of *Eucalyptus camaldulensis* under laboratory conditions. *Eucalyptus camaldulensis* 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 windbreak and medical plant. In studied area which consisted 6000 hec, many plants (range species, cultivatable and medical plant) were cultivated based on different goals. This research was conducted to increase the resistance of *Vicia villosa* in facing inhibitory effect of *Eucalyptus camaldulensis* by using chemical stimulators (include: gibberellic acid and salicylic acid as pre-treatment).

MATERIAL AND METHODS

This research was conducted to determine the effect of chemical stimulators used to reduction of allelopathic effect of *Eucalyptus camaldulensis* on germination and primary growth of *Vicia villosa*. To meet this aim, at first, aerial and underground parts of *Eucalyptus camaldulensis* were collected from Chah Nime, Zabol, Iran. After air drying at room temperature, 5 g of powder was obtained and mixed in 100 mL water, placed on a shaker for 24 h then centrifuged at 3000×g 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 *Vicia villosa* (collected from Chah Nime's farm and rangeland) were disinfectant using 5% solution of sodium hypochlorite before starting of test and were washed 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 was used as control treatment simultaneously. All seeds were washed with distilled water after soaking period and then were placed into petridishes 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 *Eucalyptus camaldulensis*. Petridishes were sterile 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 Petridishes) in different concentration of extract related to *Eucalyptus camaldulensis* (0, 5, 25, 50 and 75 percent) 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 vigority index of seed were measured.

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

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

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

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

where S_i: number of germinated seed at each counting, D_i: number of day until n counting, n: numbers of counting.

$$plant\ length = root\ length + shoot\ length$$

$$V_i = \frac{Gr_{\%} \times MSH}{100}$$

where V_i vigority index, *MSH*: mean of plant length (*root length* + *shoot length*) [mm], $Gr_{\%}$ Germination percentage

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

RESULTS

Results of variance analysis (Table 1) showed that chemical stimulators and various concentrations of *Eucalyptus camaldulensis* had significant effect on all studding properties of *Vicia villosa* species at 1% statistical level. Also interaction of chemical stimulators and various concentrations of extract had significant effect studding properties except for shoot length at 0.05 % statistical level.

Table 1

Variance analysis of studied traits of *Vicia villosa*

Properties		A	B	A × B	Error
Germination percentage	df	6	4	24	105
	ss	14084.3	10707.1	2922.9	7825.0
	ms	2347.4	2676.8	121.8	74.5
	F	31.5**	35.9**	1.6*	-
Germination speed	df	6	4	24	105
	ss	171.9	117.7	15.1	21.2
	ms	28.6	29.4	0.6	0.2
	F	141.8**	145.6**	3.1**	-

Table 1

Continued

Properties		A	B	A × B	Error
Root length	df	6	4	24	105
	ss	403.4	538.1	45.9	33.4
	ms	67.2	134.5	1.9	0.3
	F	210.7**	421.7**	5.9**	-
Shoot length	df	6	4	24	105
	ss	165.0	196.4	12.8	31.3
	ms	27.5	49.1	0.5	0.2
	F	92.0**	164.3**	1.7*	-
Plant length	df	6	4	24	105
	ss	1056	1381.1	58.4	75.5
	ms	176.0	345.2	2.4	0.7
	F	244.6**	479.9**	3.3**	-
Seed vigor	df	6	4	24	105
	ss	9535540.9	14412756.5	819220.8	1199286.8
	ms	1589256.8	3603189.1	34134.2	11421.8
	F	139.1**	315.5**	3.0**	-

**: significant differences between treatments at 1% level; ns: nonsignificant differences between treatments; A = pretreatment; B = allelopathic extract

Germination percentage and speed

The results of this research showed that increasing of various concentrations of allelopathic extract of *Eucalyptus camaldulensis* caused reduction of germination percentage of *Vicia villosa* seeds. Differences were significance between control treatment and various concentrations of extract. All the chemical stimulators could increase germination percentage of *Vicia villosa* seeds comparing to control treatment (Fig. 1) so that maximum of germination percentage was related to various concentrations of gibberelic acid. Interaction effects of chemical stimulators and various concentrations of *Eucalyptus camaldulensis* on germination percentage of *Vicia villosa* seeds were significance at 1% level (Fig. 1). Results showed that germination speed of seeds that were exposure of various concentrations of extracts had significant differences with the control treatment. Using of chemical

stimulators doesn't have any effect on germination speed so that they decreased germination speeds compare to control treatment (Fig. 2).

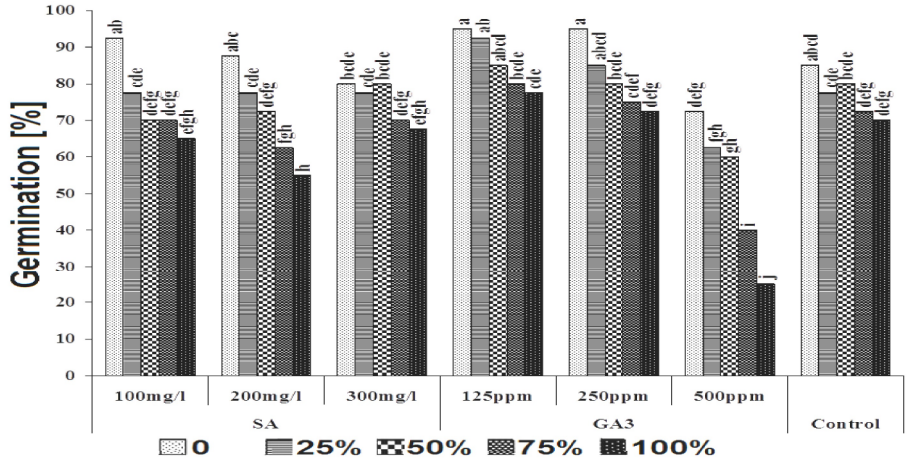

Fig. 1. Interaction comparison of chemical stimulators and various concentrations on germination of *Vicia villosa*

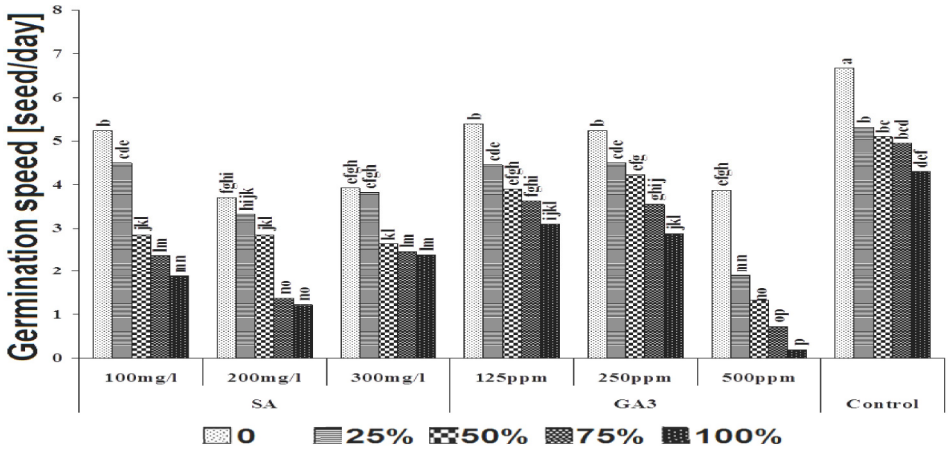

Fig. 2. Interaction comparisons of chemical stimulators and various concentrations on germination speed of *Vicia villosa*

Root, shoot and plant length

Interaction effect of chemical stimulators and various concentrations of extract of *Eucalyptus camaldulensis* were significance on root length. All stimulators improved root length in stress condition with extract of *Eucalyptus camaldulensis* so that the highest root length was related to use of various concentration of gibberellic acid (Fig. 3).

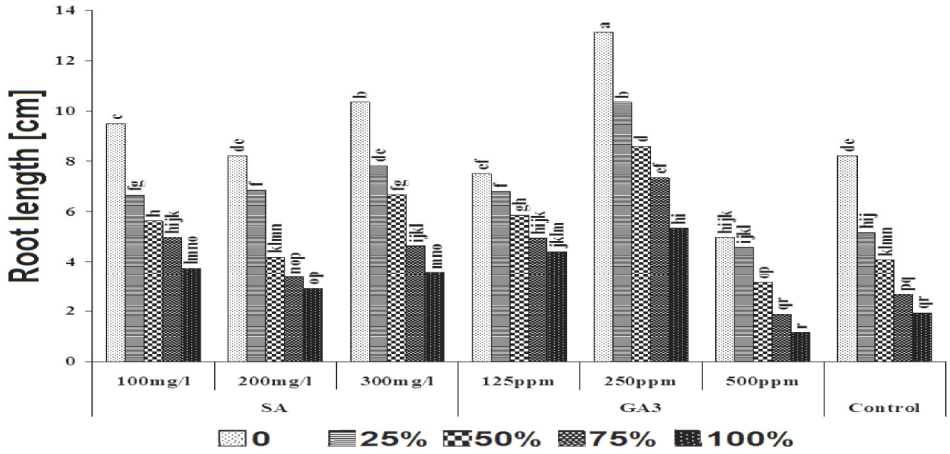

Fig. 3. Interaction comparison of chemical stimulators and various concentrations on root length of *Vicia villosa*

Results also indicated that Interaction effects of chemical stimulators and various concentrations of extract of *Eucalyptus camaldulensis* were significance on shoot length. The highest shoot length of *Vicia villosa* was related to use of gibberllic acid treatment in stress and non stress conditions. Various concentrations of *Eucalyptus camaldulensis* reduced shoot length of the species. Chemical stimulators caused an increase in shoot length in stress condition which differences were significance (Fig. 4).

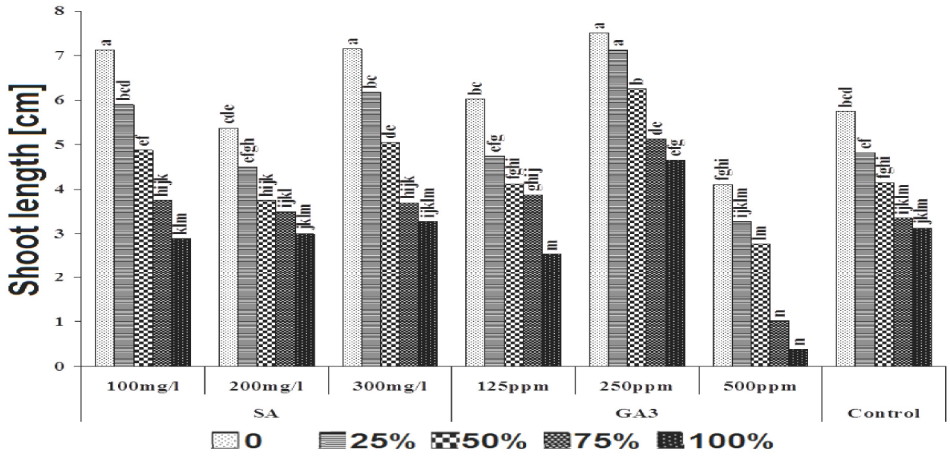

Fig. 4. Interaction comparison of chemical stimulators and various concentrations on shoot length of *Vicia villosa*

Mean comparison of data showed that interaction effect of chemical stimulators and various concentrations of *Eucalyptus camaldulensis* were significant on plant length so that plant length reduced by increasing of

concentration of *Eucalyptus camaldulensis*. In reverse all concentrations of chemical stimulators caused an increase in plant length of *Vicia villosa* in stress condition. The highest its plant length was related to use of gibberellic acid at 250 ppm in stress and non stress conditions (Fig. 5).

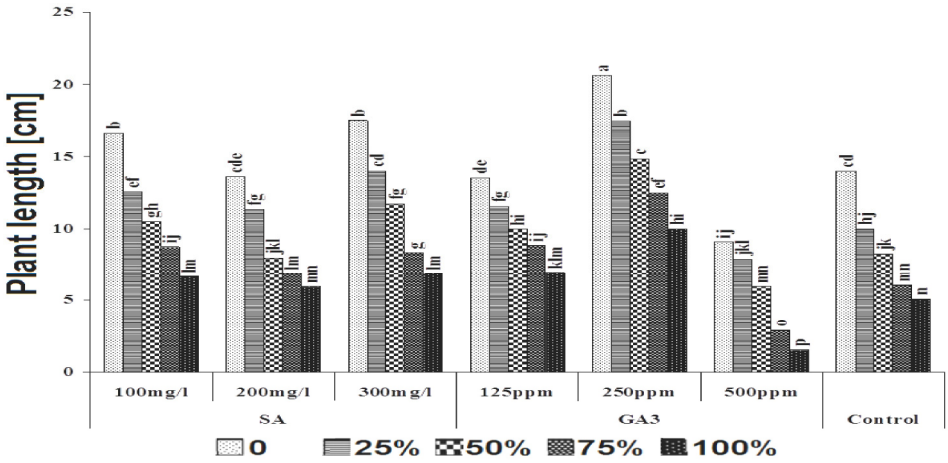

Fig. 5. Interaction comparison of chemical stimulators and various concentrations on plant length of *Vicia villosa*

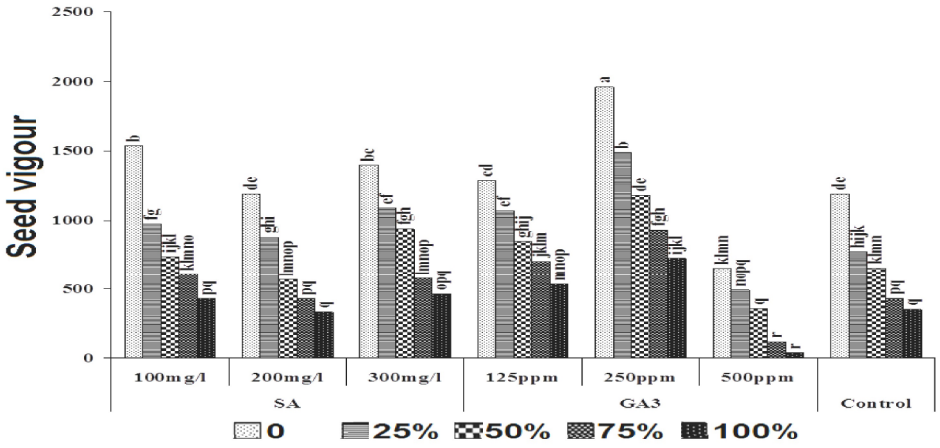

Fig. 6. Interaction comparison of chemical stimulators and various concentrations on seed vigor of *Vicia villosa*

Seed vigor index

Mean comparison showed that interaction effect of chemical stimulators and various concentrations *Eucalyptus camaldulensis* were significance on vigority index. Results showed that vigority index reduced by increasing the concentration of

Eucalyptus camaldulensis and it was significance by comparison to the control treatment. In reverse chemical stimulators increased vigority of seeds as the highest increase was related to gibberellic acid at 250 ppm (Fig. 6).

DISCUSSION AND CONCLUSION

The aim of this research was to test effects of pretreatment (salicylic and gibberellic acid) on germination properties of *Vicia villosa* under stress. Results of these research showed that chemical stimulators used in this study plays key role in reduction of stress that induced by allelopahic compounds of *Eucalyptus camaldulensis*. This results are coincide with the results of Saberi *et al.* (2011) who stated chemical stimulators such as gibberellic and salicylic acid could be used as stimulator for improving germination under allelopathic condition. Obtained results from Kang and Saltveit (2002) and Tasgin *et al* (2003) also verify this hypothesis that salicylic acid is a suitable stimulator for seed germination. Salicylic acid increases the germination by neutering of free radicals or active oxygen (Hus and Sung, 1997), increasing of antioxidant such as ascorbate (Baalbaki *et al.,* 1999), decreasing of ion transfers and accumulation of toxic ions (Krantev *et al,* 2008) and increasing of some plant hormones such as auxins and cytokinins (Sharycova *et al.,* 2003). In addition to effect of salicylic acid in increasing plant growth in stress condition, This research confirms importance of this phenolic compounds on improvement of initial growth stage when seeds exposure to stress condition with *Eucalyptus camaldulensis*. Germination and early seedling growth decreased by increasing extract concentration. This may be because of inhibitive effects of allelochemical on gibberellic acid. Pretreatment with gibberellic acid increased germination and early seedling growth of *Vicia villosa* significantly under stress and non stress condition. This hormone has an important role in germination of seeds (Ritchie and Gilroy, 1998). Gibberellic acid increases 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 which exposed to allelochemicals. Disorder in respiration results limits in metabolic energy and causes decrease in germination and early seedling growth (Cirac *et al.,* 2004).

Over all, results indicated that pretreatment of seeds by gibberellic and salicylic acid cause improvement in germination properties of *Vicia villosa* under stress and non stress condition. It is suggested that 250 ppm of gibberellic acid was used as pretreatment to decrease the negative effects of *Eucalyptus camaldulensis* on *Vicia villosa.* Hence germination percentage

and establishment of *Vicia villosa* must be increased by pretreatment with gibberellic acid before planting in field.

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