

Influence of some chemical compounds on germination and early seedling growth of two range species under allelopathic conditions

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Abstract Seed priming technique has been known to improve germination and seedling emergence under different environment stresses. The objective of this study was to evaluate the effectiveness of seed priming in improving the seed germination and seedling vigor of *Agropyron elongatum* and *Bromus inermis* in response to five concentrations of the allelopathic extract of *Thymus kotschyanus* (0, 5%, 25%, 50% and 75%) under laboratory conditions. Pretreatments included gibberellic acid (250, 500 and 1000 ppm), potassium nitrate (0.1%, 0.2% and 0.3%) and salicylic acid (100, 200 and 300 mg/L). This experiment was carried out as a factorial experiment based on a completely randomized design with four replications. The results showed that seed germination, germination speed and seedling vigor were significantly decreased ($P < 0.01$) by increasing extract concentrations of *Thymus kotschyanus* allelopathic compounds in unprimed seed (control) compared with primed seeds. Seed priming enhanced germination percentage, germination speed and initial growth. Overall, the results of this study showed that the response of both species to pretreatment of seeds was positive. Therefore, pretreatments of seeds with the aforementioned chemical stimulators before planting can significantly reduce the negative effect of allelopathic compounds in *Thymus kotschyanus* during the germination stage and contribute to plant establishment by increasing germination speed and growth of embryos.

Keywords allelopathy, germination, chemical stimulators, *Thymus kotschyanus*, *Bromus inermis*, *Agropyron elongatum*

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, infiltration and function of temperature, prevention of cell division and activity of some enzymes. These compounds can also affect the equilibrium of plant hormones, pollen tube germination, absorption of nutrient elements, displacement of stomata, photosynthesis, respiration, protein synthesis, pigment, and

changes in DNA and RNA structures (Glass, 1974). Allelochemicals are secondary metabolites of organisms without nutritional value. These chemicals have inhibitory or stimulating effects on the growth, health, behavior, and biotic population of other organisms (plants, insects, microbes, etc.) (Zhang et al., 2003). Safari et al. (2010) reported that aqueous extracts of *Thymus kotschyanus* had a considerable inhibitory effect on germination of *Bromus tomentellus* and *Trifolium repens* with the effects at 50%, 75%, and 100% concentration significantly higher than that at lower concentrations (5% and 25%) and the control treatment (distilled water).

Allelopathy has an effect on plants in agricultural ecosystems through the interactions of allelochemicals directly with plants or through biological or nonbiological processes in the soil (Inderjit, 2001). Primary growth stages are strongly affected by these compounds. Inhibition of germination may be caused by changes in the activities of

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enzymes that affect the transmission of reserve compounds during the germination stage (El-Khatib et al., 2004). Disorders in respiration amount can also lead to restrictions in metabolic energy and would therefore reduce germination and plant growth. This reduction can lead to many effects, for example, bigger plants have a more competitive advantage over nearby plants under adverse conditions such as low soil moisture or nutrient limitations. Also, delayed seed germination can create osmotic effects on the rate of water absorption and especially cell elongation (Escudero et al., 2000).

Seed priming is used in germination improvement, reduction of germination time and embryo emergence, and improvement of establishment and performance. Priming is also applied to increase seed vigor and reduce losses from late plantation. Fast germination is affected by priming that arises from DNA, RNA and protein synthesis (Barsa et al., 2003). Many researchers have reported that priming can increase germination percentage and emergence in weakened or damaged seeds (Horii et al., 2007).

Different physiological and biochemical effects of salicylic acid on plant systems have been observed, including ion absorption, membrane permeability, mitochondrial respiration, closure of stomatal transmission of materials, growth rate and photosynthesis rate (Senaratna et al., 2000). Furthermore, the effect of salicylic acid on many physiological processes in cells has been reported (Zhang et al., 2003). There is some evidence that seed treatment with salicylic acid can increase some plant hormones, such as oxynes and sytocenines (Sharikova et al., 2003), and reduce ion leakage from plant cells (Balibrea et al., 2000; Borsani et al., 2001; Ghoulam et al., 2002). Seed germination is initiated by water absorption followed by biochemical events in the seed (Greipsson, 2001) including activation of metabolism, digestion of reserve compounds and transmission to embryos, cell division and growth (Albeles and Lonski, 1969). It is known that gibberellic acid has the main role in these processes. Chemical compounds that penetrate into the embryo and stimulate metabolic activities often are effective in stimulating germination. Potassium nitrate stimulates many light-sensitive seeds in the dark. However, its effect is influenced by a variety of factors.

Pretreatment of seeds is a simple technique that is a low-cost and low-risk solution to improve seed germination and growth of embryos.

The objective of this study was to evaluate the effectiveness of seed priming in improving seed germination and seedling vigor of *Agropyron elongatum* and *Bromus inermis* in response to the allelopathic effects of *Thymus kotschyanus* under laboratory conditions. *T. kotschyanus* is a known pharmaceutical plant that is almost impossible to eliminate in the study area. Hence, this research was conducted to increase the resistance of two species (*B. inermis* and *A. elongatum*) when faced with the inhibitory effect of *T. kotschyanus* allelopathic compounds by pretreatment using the chemical

stimulators gibberellic acid, salicylic acid and potassium nitrate.

Materials and methods

Aerial and underground parts of *T. kotschyanus* were collected from Arton in Taleghan, 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 5, 25, 50 and 75% were prepared using the centrifuged solution. Seeds of *A. elongatum* and *B. inermis* were collected from Taleghan in the summer of 2009. They were first disinfected using a 10% solution of sodium hypochlorite then washed with distilled water several times. The disinfected seeds were pretreated with either salicylic acid at 100, 200 and 300 mg for 72 h potassium nitrate at 0.1%, 0.2% and 0.3% for 24 h or gibberellic acid at 250, 500 and 1000 ppm at 25°C. Distilled water was used as the control treatment. All seeds were washed with distilled water after the soaking period, dried and then placed into Petri dishes with a 9 cm filter paper (Whatman 1) in order to test different stress conditions with various concentrations of the allelopathic extract of *T. kotschyanus*. Petri dishes were first sterilized for 48 h in the oven at 20°C before placing the seeds. The germination test was performed using a completely randomized factorial design with four replications (25 seeds per replication) with different concentrations of the extract of *T. kotschyanus* (0, 5, 25, 50 and 75%) at 25°C in the germinator. Germinated seeds of more than 2 mm length were counted each day over 10 d (Kaya et al., 2006) and the germination percentage, germination speed, root length, shoot length, plant length and seed vigor index were measured.

Germination percentage and germination speed were calculated based on the following equations:

(1) Germination percentage:

$$Gp = \frac{\sum G}{N} \times 100,$$

where Gp is germination percentage, G is the number of germinated seeds and N is the number of seeds.

(2) Germination speed:

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

where S_i is the number of germinated seed at each counting, D_i is the number of day until n counting and n is the number of counting.

(3) Vigor index = Total germination percentage × Plant length.

(4) Plant length = Root length + Shoot length.

The collected data were analyzed using MSTAT-C and mean comparison was performed using Duncan's Multiple Range Test.

Results

Variance analysis showed that chemical stimulators and various concentrations of the allelopathic extract of *T. kotschyanus* had significant effects on all the studied traits of *A. elongatum* and *B. inermis* species at 1% statistical level. Moreover, the interaction of chemical stimulators and various concentrations of the allelopathic extract did not have a significant effect on the germination percentage of *A. elongatum* whereas the difference was significant in the other traits (Tables 1 and 2).

Germination percentage and germination speed

As shown in Figures 1 and 2B, the increasing concentrations of the allelopathic extract of *T. kotschyanus* caused the reduction of germination percentages of *A. elongatum* and *B. inermis* seeds. The differences were significant between the control treatment and various concentrations of the extract. All the chemical stimulators increased the germination percentage of *A. elongatum* seeds compared with the control treatment (Fig. 2A), however, pretreatment with potassium nitrate did not have any effect on increasing the germination percentage of *B. inermis* compared to the control treatment. The highest germination percentages in both species were observed in the different concentrations of salicylic acid and

gibberellic acid. The interaction of chemical stimulators and various concentrations of the allelopathic extract of *T. kotschyanus* on germination percentage of *A. elongatum* seeds was not significant, but this interaction was significant in *B. inermis* at 5% level (Fig. 1). However, the interaction of chemical stimulators and various concentrations of the allelopathic extract of *T. kotschyanus* was significant for the germination rates of *A. elongatum* and *B. inermis* seeds. Results showed that the germination speed of seeds at various concentrations of the allelopathic extract was significantly different compared to the control seeds. The increasing concentrations of the allelopathic extract reduced the germination speed in both species (Figs. 3 and 4). All three chemical stimulators increased the germination speed of *A. elongatum* seeds. The highest germination rates for *A. elongatum* and *B. inermis* were observed in different concentrations of gibberellic acid, while no significant effect on the germination rate was found in the pretreatment with potassium nitrate compared to the control treatment. Chemical stimulators improved the germination rate of *A. elongatum* seeds under both stress and nonstress conditions.

Root length, shoot length and plant length

The interaction of chemical stimulators and various concentrations of the allelopathic extract of *T. kotschyanus* on root

Table 1 Variance analysis of studied traits of *A. elongatum*

Properties		A	B	A*B	Error
Germination percentage	f	9	4	36	150
	ss	462.7	2013.9	83.68	11.26
	ms	51.4	503.5	2.32	0.07
	F	4.5**	44.95**	0.21ns	
Germination speed	f	9	4	36	150
	ss	1150.5	299.16	35.8	11.3
	ms	127.8	74.8	0.99	0.07
	F	1697.5**	993.1**	13.2**	
Root length	f	9	4	36	150
	ss	79.6	205.9	17.7	7.2
	ms	8.8	51.5	0.5	0.05
	F	185.3**	1077.9**	10.3**	
Shoot length	f	9	4	36	150
	ss	175.2	155.4	30.3	12.5
	ms	19.6	38.9	0.84	0.08
	F	234.0**	464.6**	10.1**	
Plant length	f	9	4	36	150
	ss	467.8	713.9	64.2	26.3
	ms	52.0	178.5	1.8	0.17
	F	296.9**	1019.4**	10.2**	
Seed vigor	f	9	4	36	150
	ss	3974515.5	7474770.1	514683.3	432037.4
	ms	141612.8	1868692.5	1867692.5	2880.2
	F	153.3**	648.8**	4.9**	—

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

Table 2 Variance analysis of studied traits of *B. inermis*

Properties		A	B	A* B	Error
Germination percentage	f	9	4	36	150
	ss	30919.4	17820.8	3253.8	1284.750
	ms	3435.5	4455.2	90.4	8.565
	F	401.1**	520.2**	10.5**	
Germination speed	f	9	4	36	150
	ss	1186.9	420.8	46.1	0.032
	ms	131.9	105.2	1.3	0.000
	F	610594.9**	487077.5**	5927.4**	
Root length	f	9	4	36	150
	ss	282.7	691.1	79.1	5.5
	ms	31.5	172.8	2.2	0.037
	F	856.3**	4710.3**	59.9**	
Shoot length	f	9	4	36	150
	ss	17.2	179.9	41.5	1.3
	ms	1.9	44.9	1.1	0.009
	F	218.7**	5140.1**	131.8**	
Plant length	f	9	4	36	150
	ss	325.8	1575.9	170.5	6.3
	ms	36.2	393.9	4.7	0.042
	F	862.6**	9388.1**	112.9**	
Seed vigor	f	9	4	36	150
	ss	5333000.9	14803545.5	1192521.8	91641.6
	ms	592555.6	592555.6	33125.6	610.9
	F	969.9**	6057.6**	54.2**	

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

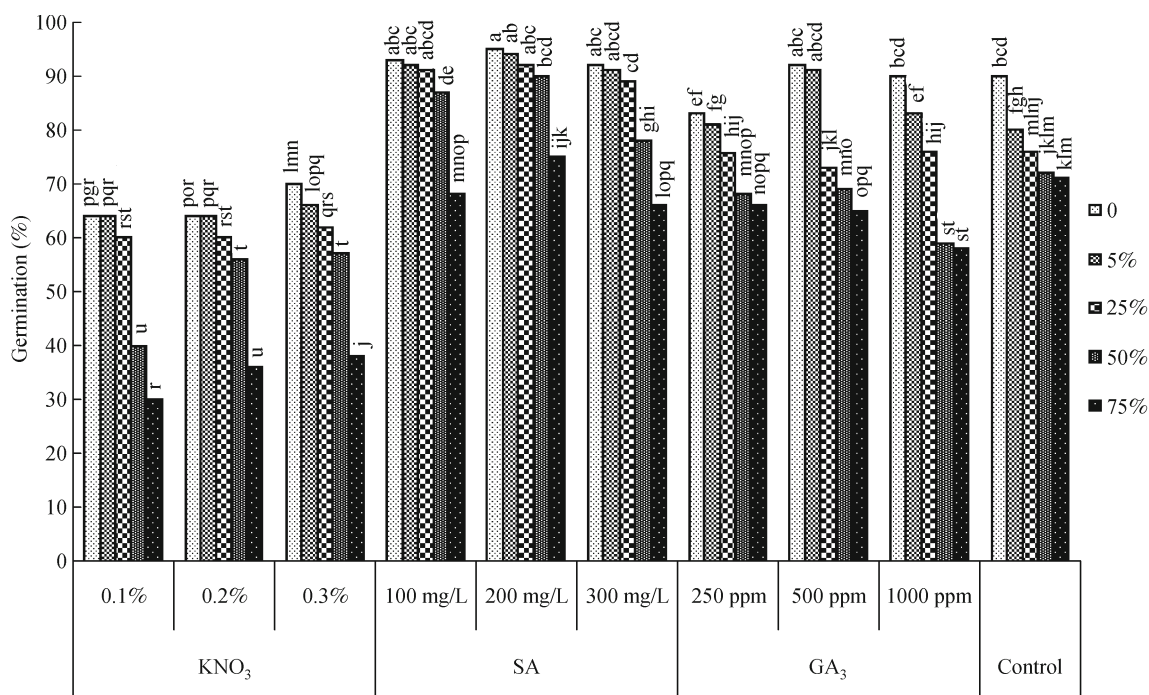


Figure 1 Interaction comparison of chemical stimulators and various concentrations on germination percentage of *B. inermis*. Significant differences indicated by different letters ($P < 0.05$).

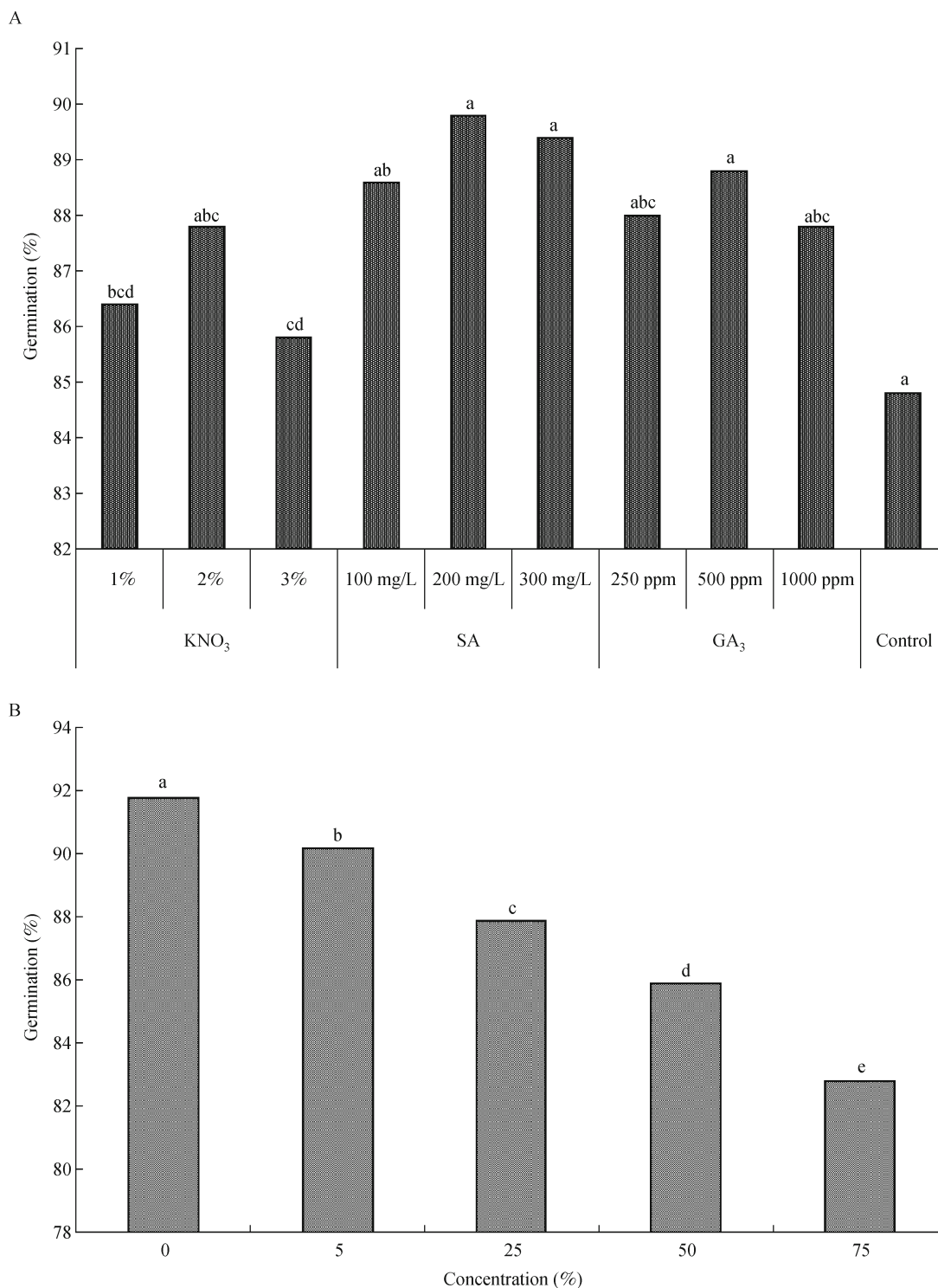


Figure 2 Effects of chemical stimulators (A) and allelopathy of *T. kotschyanus* (B) on germination percentage of *A. elongatum*. Significant differences indicated by different letters ($P < 0.05$).

length was also significant. All of the stimulators improved root length under stress conditions with the allelopathic extract of *T. kotschyanus* and the highest root lengths were observed in the different concentrations of gibberellic acid and salicylic acid. Results showed that chemical stimulators

improved root length under stress and non-stress conditions (Figs. 5 and 6).

The highest shoot length of *A. elongatum* under stress and non-stress conditions was found in the gibberellic acid treatments, but in *B. inermis* it was observed in the potassium

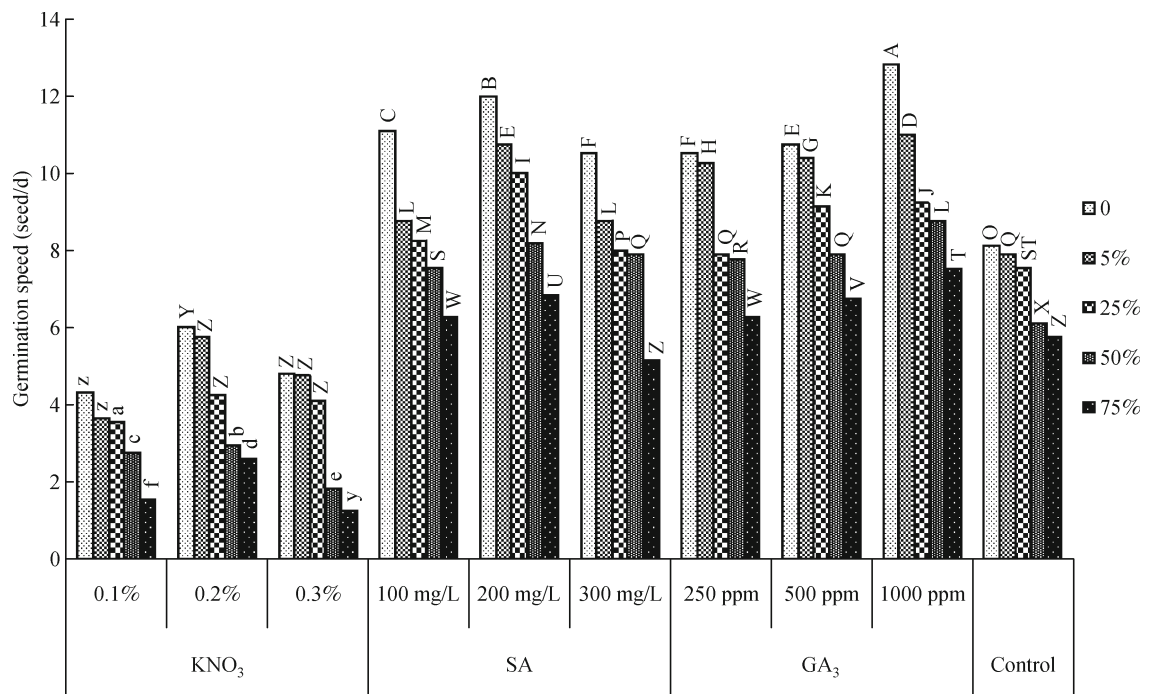


Figure 3 Interaction comparison of chemical stimulators and various concentrations on germination speed of *B. inermis*. Significant differences indicated by upper-case letters (A, B, etc.) and followed by lower-case letter (a, b, etc.). Significant differences indicated by different letters ($P < 0.05$).

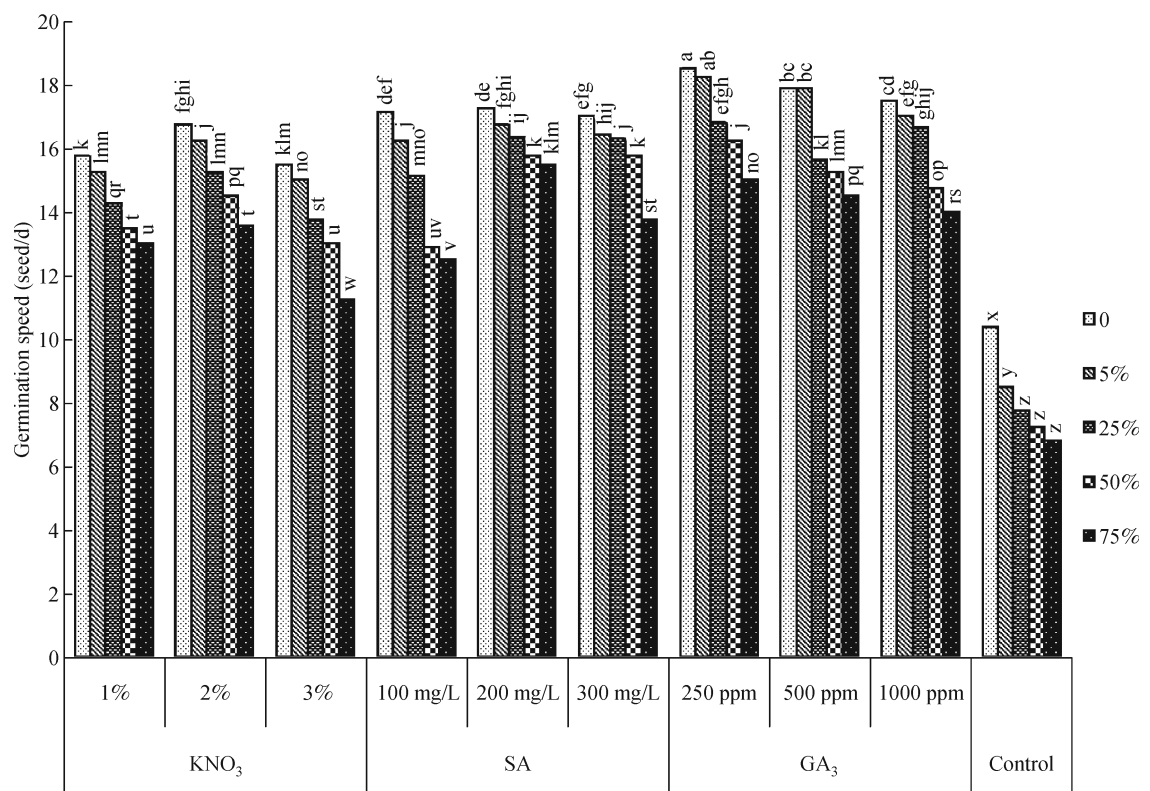


Figure 4 Interaction comparison of chemical stimulators and various concentrations on germination speed of *A. elongatum*. Significant differences indicated by different letters ($P < 0.05$).

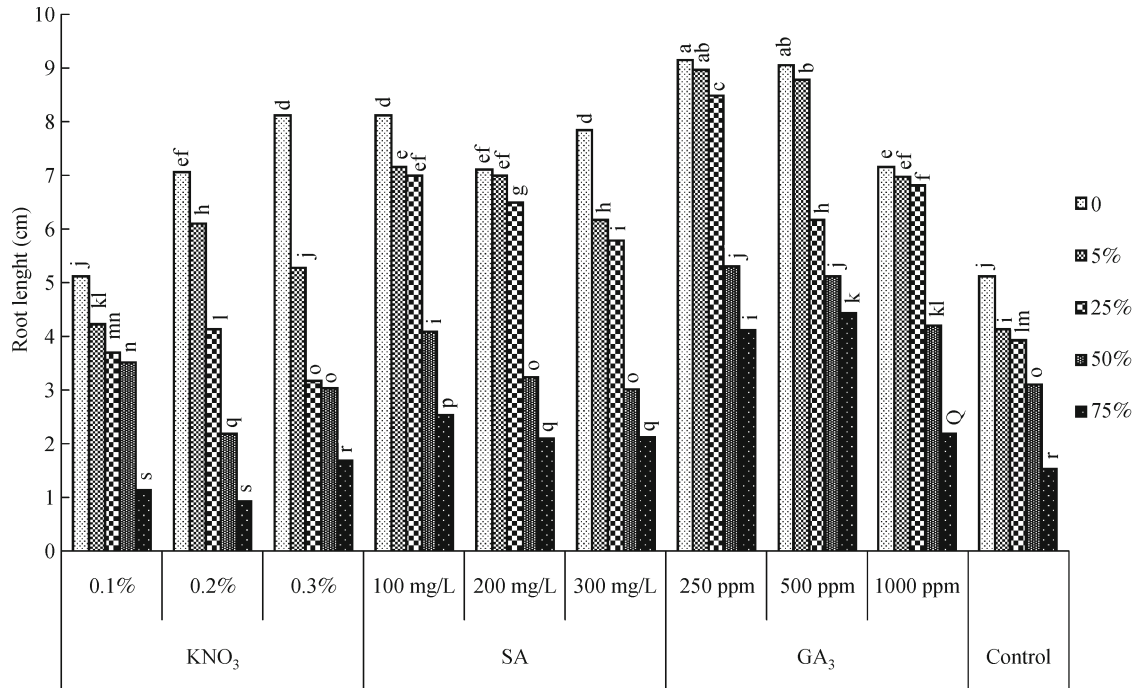


Figure 5 Interaction comparison of chemical stimulators and various concentrations on root length of *B. inermis*. Significant differences indicated by different letters ($P < 0.05$).

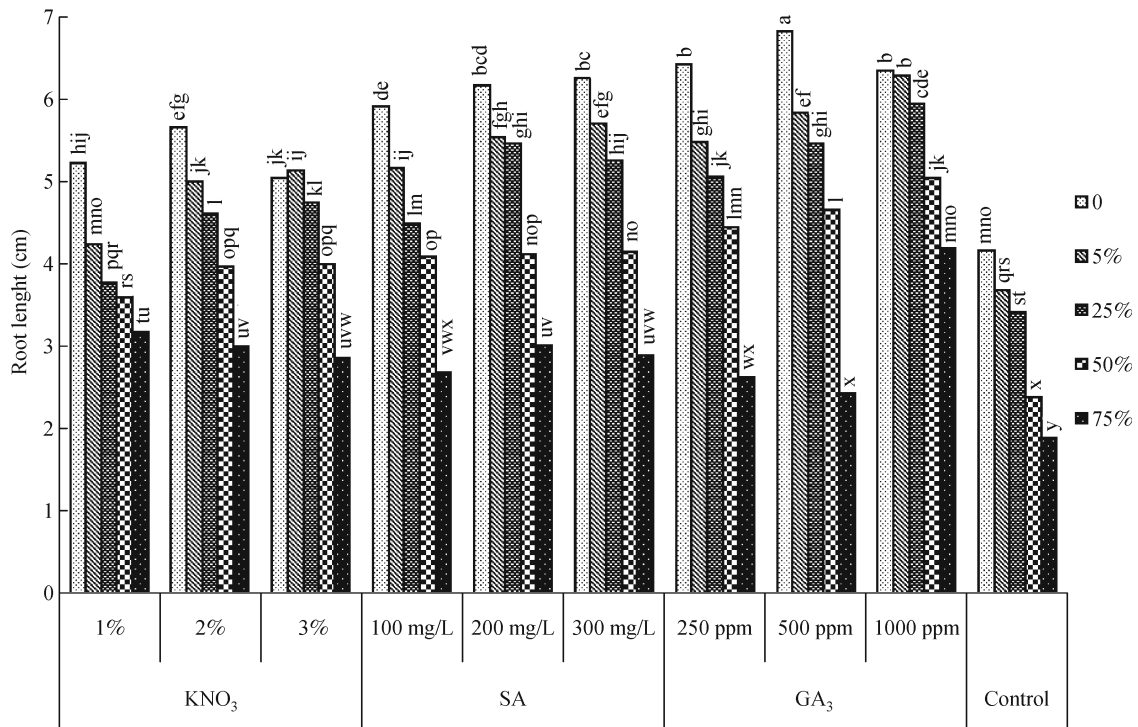


Figure 6 Interaction comparison of chemical stimulators and various concentrations on root length of *A. elongatum*. Significant differences indicated by different letters ($P < 0.05$).

nitrate treatments. Various concentrations of *T. kotschyanus* allelopathic extract reduced the length of shoot and plants of the two species. All chemical stimulators in all concentrations

significantly increased plant length under stress conditions of the allelopathic extract of *T. kotschyanus* (Figs. 7 and 8).

As shown in Figs. 9 and 10, the mean comparison of the

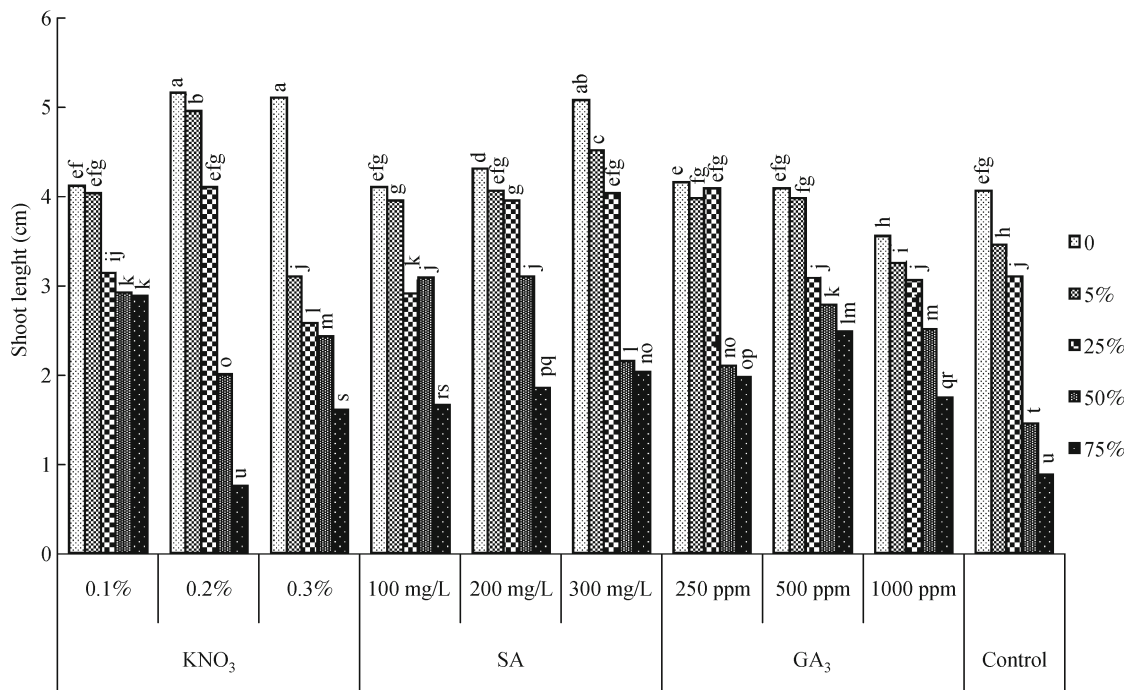


Figure 7 Interaction comparison of chemical stimulators and various concentrations on shoot length of *B. inermis*. Significant differences indicated by different letters ($P < 0.05$).

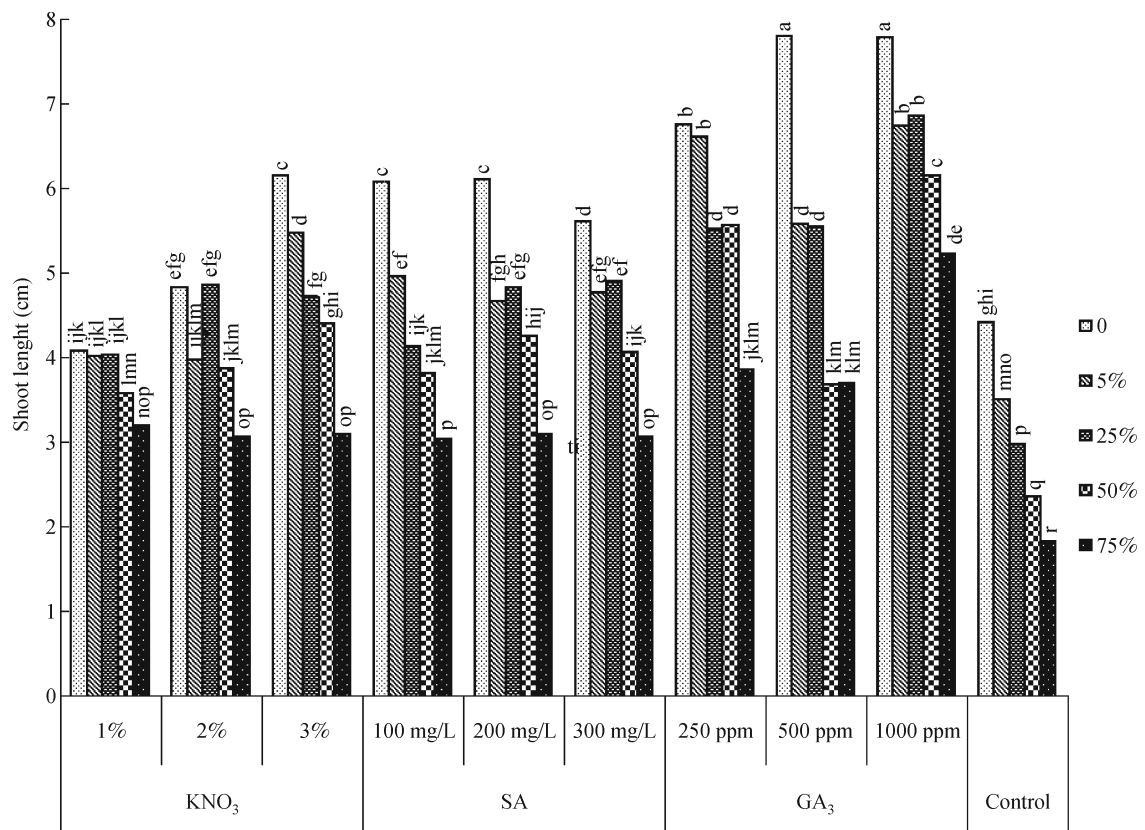


Figure 8 Interaction comparison of chemical stimulators and various concentrations on shoot length of *A. elongatum*. Significant differences indicated by different letters ($P < 0.05$).

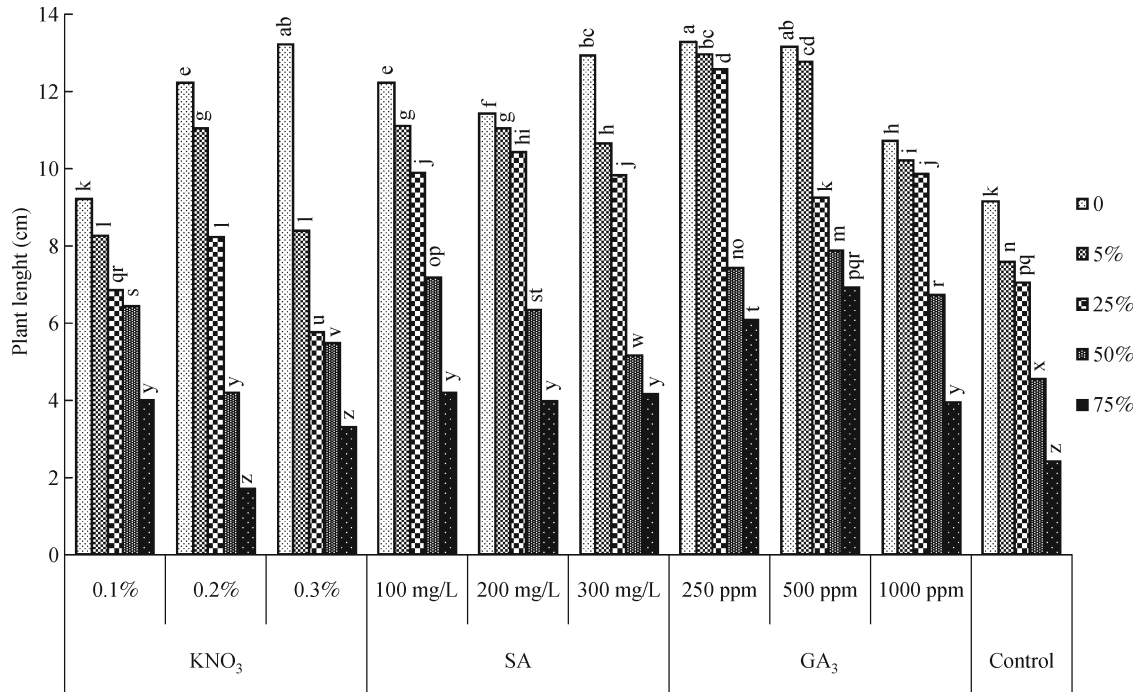


Figure 9 Interaction comparison of chemical stimulators and various concentrations on plant length of *B.inermis*. Significant differences indicated by different letters ($P < 0.05$).

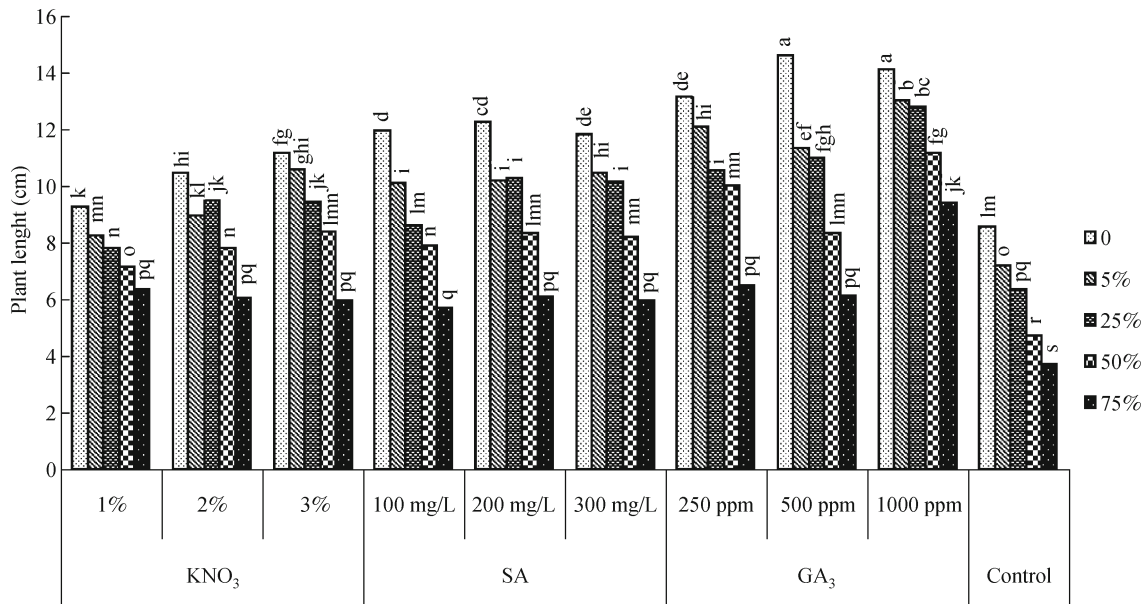


Figure 10 Interaction comparison of chemical stimulators and various concentrations on plant length of *A. elongatum*. Significant differences indicated by different letters ($P < 0.05$).

interaction of chemical stimulators and various concentration of the allelopathic extract of *T. kotschyanus* on plant length of both species was significant. Such that with increasing concentrations of the *T. kotschyanus* extract, plant length was reduced in both *A. elongatum* and *B. inermis*. In contrast, chemical stimulators in all concentrations caused a significant increase in plant length under stress conditions of the

allelopathic extract of *T. kotschyanus*.

Seed vigor index

The mean comparison shows that the interaction of chemical stimulators and various concentrations of *T. kotschyanus* allelopathic extract on vigor index was significant in both *A.*

elongatum and *B. inermis*, indicating that vigor index was significantly reduced with increasing concentrations of the *T. kotschyanus* extract, compared to the control group. In contrast, the chemical stimulators in three concentrations increased seed vigor index of *A. elongatum* and *B. inermis*. The highest increase for both two species was observed in different concentrations of gibberellic acid and salicylic acid. Therefore, chemical stimulators could improve the vigor index of seeds under both stress and non-stress conditions (Figs. 11 and 12).

Discussion and conclusions

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 *T. kotschyanus* on the primary growth of *A. elongatum* and *B. inermis* embryos. The use of the hormone gibberellic acid significantly increased germination rate, root length, shoot length, plant length, and seed vigor index of *A. elongatum* and *B. inermis* under stress and non-stress conditions caused by the allelopathic compounds of *T. kotschyanus*. Plant hormones such as gibberellic acid have an important role in the germination process (Ritchie and Gilroy, 1998). Gibberellic acid produced during germination is used directly in plant growth through

hydrolysis of storage foods (Kepczynski and groot, 1989). External use of gibberellic acid on seeds causes the breaking of seed dormancy (Dunand, 1992). One reason for the positive effect of chemical stimulators such as gibberellic acid and potassium nitrate on the primary growth of *A. elongatum* and *B. inermis* embryos is probably related to achieving hormonal balance in the seed and the reduction of inhibitory substances such as abscisic acid (ABA). Gibberellins were reported to increase the synthesis of hydrolytic enzymes located under the aleurone layer. These synthesized enzymes were then transported to the endosperm, causing the breakdown of reserved food leading to a supply of energy for germination and growth (Çirak et al., 2004). In our research, salicylic acid increased the primary growth of *A. elongatum* and *B. inermis* under stress and non-stress conditions from the allelopathic compounds of *T. kotschyanus*. Kang and Saltveit (2002) and Taşğın et al. (2003) have suggested that salicylic acid was a proper stimulator for germination and growth and produced reactive oxygen species (ROS) as well as resistance in plants. Furthermore, salicylic acid increased the levels of some plant hormones, such as auxins and cytokinins (Sharikova et al., 2003), and significantly reduced ion leakage and accumulation of toxic ions in plants (Krantev et al., 2008), while influencing the removal of oxidative damage during germination (López et al., 2000). This research confirmed that phenolic compounds are very important at the initial growth stage when exposed to stress from *T. kotschyanus* allelopathic compounds and the pretreatment of seeds with salicylic acid can compensate for the reduction in growth caused by the allelopathic compounds of *T. kotschyanus*.

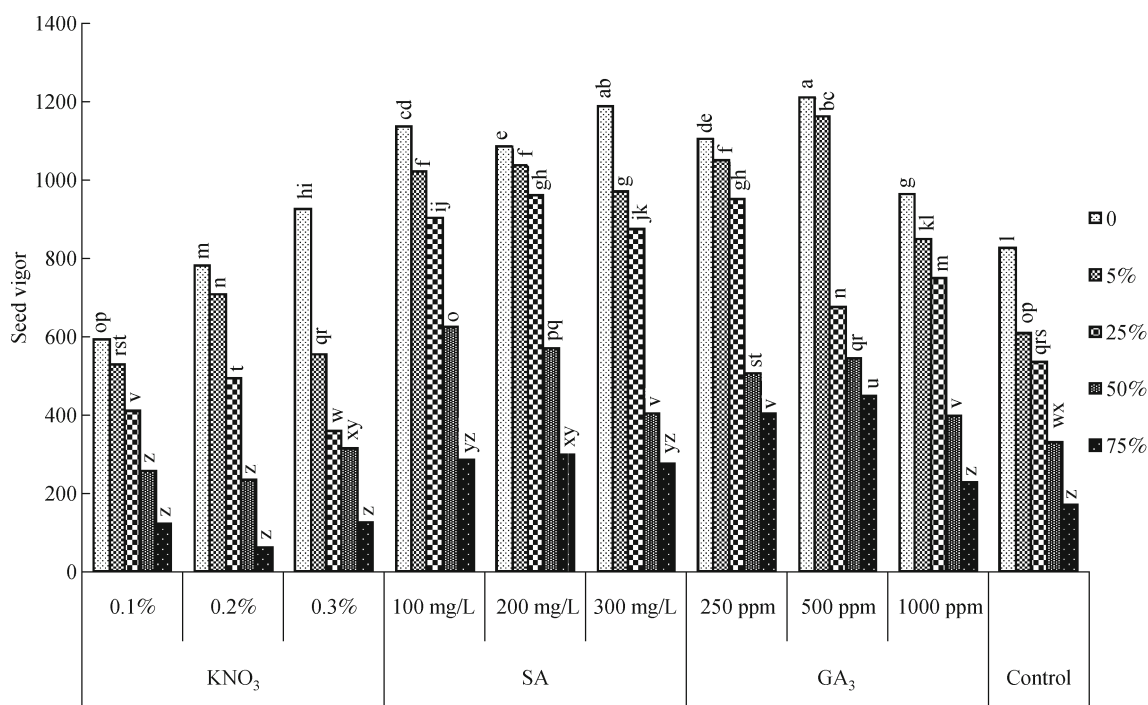


Figure 11 Interaction comparison of chemical stimulators and various concentrations on seed vigor of *B. inermis*. Significant differences indicated by different letters ($P < 0.05$).

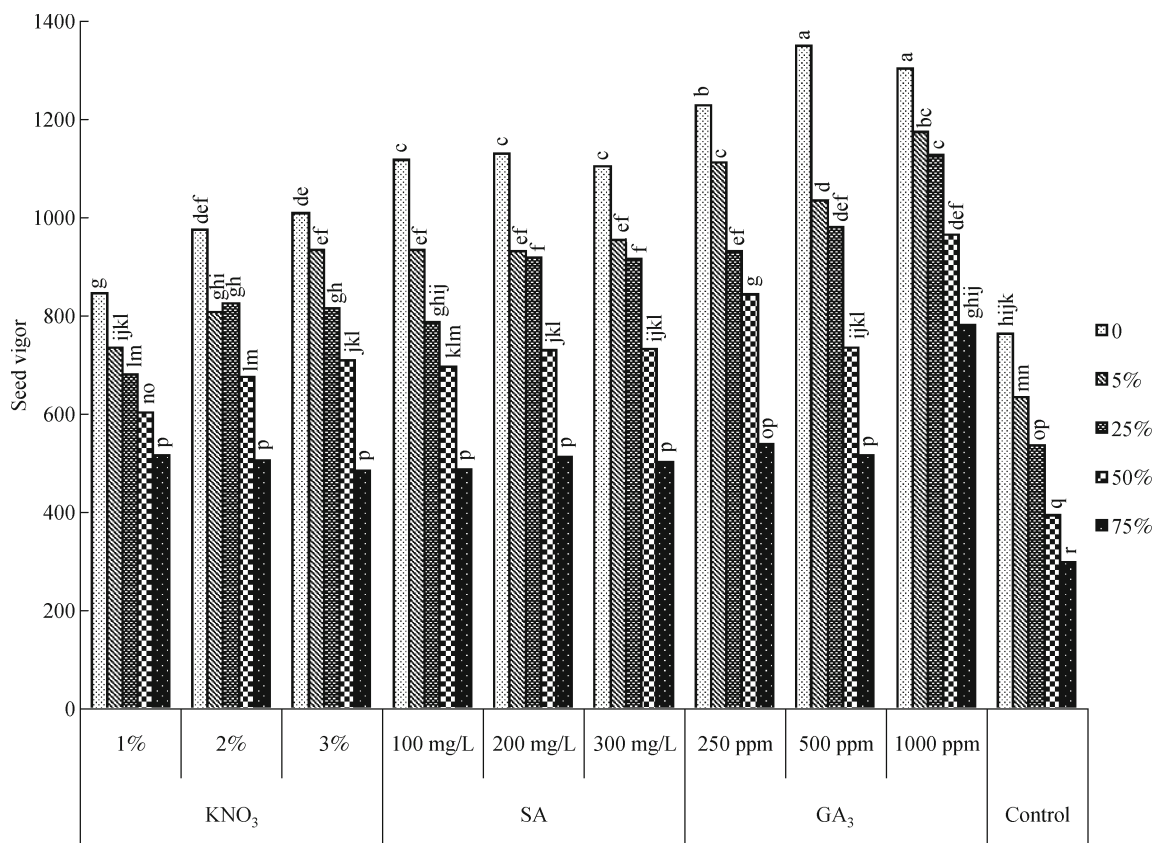


Figure 12 Interaction comparison of chemical stimulators and various concentrations on seed vigor of *A. elongatum*. Significant differences indicated by different letters ($P < 0.05$).

The pretreatment of seeds before germination showed that these compounds were transferred into seeds, to protect the development of the embryos and the stability of the plant against the allelopathic compounds of *T. kotschyanus* and there was a mutual relationship between the chemical stimulators and allelopathic compounds of *T. kotschyanus*. Salicylic acid may provide protection against certain abiotic stresses like heat stress in mustard seedlings (Dat et al., 1998), and chilling damage to maize (Janda et al., 2000), and reduce the inhibitory effect of heavy metals on some growth factors (Pál et al., 2002; Dražić et al., 2006; Popova et al., 2008).

In conclusion, chemical growth stimulators can reduce the inhibitory effects of the allelopathic compounds of *T. kotschyanus* on the seed germination and seedling growth of *A. elongatum* and *B. inermis*. To improve and recover rangelands, the use of chemical stimulators, especially gibberellic acid and salicylic acid, to decrease the inhibitory effects of the allelopathic compounds of *T. kotschyanus* is suggested.

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