

## EFFECTS OF THE ENVIRONMENTAL CHARACTERS ON GERMINATION PROPERTIES OF SEEDS OF *THYMUS DAENENSIS* AND *T. VULGARIS*

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**Abstract:** This research was conducted to investigate the effects of salinity, temperature, pH and sowing depth on germination characters of two species of thyme in 2017. Two separate experiments with four replications using a randomized complete design were conducted in laboratory conditions. Treatments contained: a) fluctuating temperatures in a germinator 5/15, 10/20, 15/25, 15/30 and 20/35<sup>0</sup>C (day/night), b) concentrations of salinity by using solutions of 0, 10, 20, 40, 80, 160 and 320 mM NaCl, c) sowing depths of 0, 1.5, 3 and 6 cm, pH values of 5, 6, 7, 8 and 9. Results showed that the effects of different treatments were significant on germination percentage, germination rate and seed vigour. In most cases, upper concentrations of treatments had negative effects on germination indices. The emergence decreased with an increased concentration of salinity, planting depth and pH. In both species, the highest germination percentage (94%) was obtained in the combination treatment of 15/25<sup>0</sup>C (day/night), 0 mM of NaCl, 1 cm of planting depth and pH=7. Germination was stopped in the combination of treatments of 35/20 (day/night), 320mM of NaCl, 6 cm of planting depth and pH=9. The combination of treatments in the upper level had a more inhibitory and destructive effect than single treatments.

**Key words:** pH, salinity, sowing depth, temperature, thyme.

### Introduction

Thyme, one of the most important spices, is used all over the world, and includes many species. Two of the most important commercially grown species are *Thymus vulgaris* L. and *T. daenensis* Celak, members of the family Lamiaceae. *Thymus* species are commonly used as herbal teas, flavouring agents (condiments and spices) and medicinal plants (Hudaib and Aburjai, 2007). The major components are phenols (mainly thymol and carvacrol), monoterpen hydrocarbon and alcohol that have insecticidal activity. Among these, thymol and carvacrol are the main compounds (Yadegari, 2015a). Thyme species are commonly used as

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flavouring agents and medicinal plants (Chauhan and Johnson, 2008; Yadegari, 2017a, b). Seeds of this plant have no dormancy (Yadegari, 2015b). Thyme morphological diversity can affect ecological factors of plant competition, time of germination, flowering time and genetic effects (Corticchiato et al., 1998). Germination is a key to the success of plants in agro-ecosystems (Chauhan and Johnson, 2008; Keller and Kollmann, 1999; Hubbard et al., 2010). The affecting factors on germination and emergence are temperature, osmotic pressure of the solution, the position of the seed in the soil seed bank and soil texture. Temperature is an important environmental factor regulating germination (Ren et al., 2002). Seed germination and emergence belong to the depth in the soil. Emergence of seedling reduces more than optimum depth. The effect of pH on germination potential varied in different plants. Seed germination, in particular, appears to be extremely sensitive to soil salinity (Amiri et al., 2012). Field salinization is a growing problem worldwide and it is a major abiotic stress reducing the yield of a wide variety of crops all over the world (Bourgou et al., 2012). Some species require more acidic conditions, and some prefer alkaline or neutral pH, while some others do not show any reactions (Susko et al., 1999). Germination is a critical stage in the life cycle of plants, and often controls population dynamics, with major practical implications (Keller and Kollmann, 1999). Overall germination events are regulated by several environmental factors such as temperature, salinity, pH and moisture (Hubbard et al., 2010). There are few studies examining environmental characters on germination properties of seeds of thyme. Objectives of this study were to determine the effects of environmental characters (salinity, temperature, pH and sowing depth) on germination properties of seed germination of *T. daenensis* and *T. vulgaris* for better establishment in rangelands.

## Materials and Methods

### Laboratory experiment and conditions

To study the effects of salinity, temperature, pH and sowing depth on germination characters of two species of thyme, two separate experiments with four replications using a randomized complete design in a factorial layout were carried out. This study was conducted in 2017 in the Center of Medicinal and Aromatic Plants of Islamic Azad University Branch of Shahrekord in laboratory conditions. Seeds of thyme species were collected naturally from mountains of Chaharmahal and Bakhtiari province (Tables 1 and 2).

Treatments contained: a) fluctuating temperatures in a germinator 5/15, 10/20, 15/25, 15/30 and 20/35<sup>0</sup>C night/day; b) concentrations of salinity by using solutions of 0, 10, 20, 40, 80, 160 and 320 mM NaCl; c) sowing depths of 0, 1.5, 3 and 6 cm, and d) pH of 5, 6, 7, 8 and 9 (Chauhan and Johnson, 2008). Seeds were

placed on two moistened paper towels. After covering the seeds with a third sheet of paper, the three towels were loosely rolled to form a tube and placed in plastic bags (23×33 cm) to prevent evaporation. Seeds were observed twice daily and considered germinated when the radicle was approximately 2 mm in length (Soltani et al., 2001).

Table 1. Geographic and climatic properties in collection sites.

Average minimum temperature	Average maximum temperature	Average annual temperature	Average annual rainfall	Elevation	Geographic properties	Mountain
	°C		Mm	meter		
-1.4	24.5	12.1	516.9	3670	32010/N-50030/E	Saldaran
-4.5	17.5	9.5	875.5	3904	3205/N-16051/E	Mili
4	27.8	18.4	500	3640	31, 82 N-50, 96 E	Kallar

Table 2. Soil properties in collection sites.

Elevation	Mountain	Texture	pH	O.C	EC	B	Mn	Fe	Cu	Zn	N	K	P
				%	ds/m								
2500	Saldaran	Clay loam	7.76	1.65	0.361	1.12	8.37	3.62	0.94	0.81	0.1	254	12.7
2500	Mili	Clay	7.12	2.2	0.38	1.3	11.1	3.8	0.55	0.52	0.3	355	19.1
2500	Kallar	Clay loam	7.93	2.85	0.481	1.23	9.38	5.32	1.02	0.77	0.311	291	34.6

#### Analysis of morphological traits

To evaluate the potential salinity on seed germination reduction, the three parameters of  $x$ ,  $x_{50}$  and  $b$  were used (Chauhan and Johnson, 2008):

$$Y = a / [1 + (x/x_{50})^b] \quad \text{Eq. (1)}$$

$Y$ : germination at the salinity level of  $X$  (%),  $a$ : maximum germination (%),  $X_{50}$ : the salinity level required for 50% inhibition of maximum germination and  $b$ : a slope represents reduced germination by increasing salinity. Seed percentage, germination percentage and seed vigour were measured by the following equations (Chachalis and Ready, 2000):

$$S.P = X_1 / Y_1 + (X_2 - X_1) / Y_2 + \dots + (X_n - X_{n-1}) / Y_n \quad \text{Eq. (2)}$$

$$G.P = \text{Number of germinated seeds} / \text{Number of total seeds} \quad \text{Eq. (3)}$$

$$S.V = \text{Mean of length of seedling} \times G.P \text{ (measured with a calliper)} \quad \text{Eq. (4)}$$

### Quality of treatments

A 2-mM potassium hydrogen phthalate buffer solution was adjusted to pH 4 with 1 N HCl. A 2-mM solution of MES [2-(N-morpholino) ethane sulfonic acid] was adjusted to pH 5 or 6 with 1 N HCl or NaOH. A 2-mM solution of HEPES [N-(2-hydroxymethyl) Piperazine-N'-(2-ethanesulfonic acid)] was adjusted to pH 7 or 8 with 1 N NaOH. pH 9 buffer was prepared with 2-mM tricine [N-Tris (hydroxymethyl) methyl glycine] and adjusted with 1 N NaOH. Non-buffered deionized water (pH 6.3) was used as control. In glass bottles with height of 15 cm, the effects of seed sowing depths on plant seedling emergence were studied.

### Statistical analysis

After the normality and homogeneity test of variance, the logarithmic transformation of data was done and then all data were subjected to ANOVA using the statistical computer package SAS ver.8 and treatment means were separated using the L.S.D multiple range test at the  $P < 0.05$  level.

## Results and Discussion

The effects of treatments on germination rate, germination percentage and seed vigour in two species were significant (Tables 3 and 4). In single treatments, the highest percentage of germination temperature was in the treatment of 15/25 (day/night) with 91% (Figure 1). Salinity was significant in measured parameters. The highest percentage of germination in seeds of *T. vulgaris* and *T. daenensis* was in 0 mM treatment amounting to 91% and 94% respectively. There was no germination in both of species regarding the salinity of 320 mM sodium chloride. Decreased 50% germination occurred at the salinity of 160 mM sodium chloride (Figure 2). The percentage and germination rates of both species of thyme seeds were influenced by acidity. The highest and lowest germination was achieved at pH 7 (94%) and pH 9 (19%) respectively (Figure 3). Seedling emergence was affected by planting depth so that significant differences were observed at various depths. The statistic groups of emergence at the soil surface (86%) and at the depth of 1.5 cm (84%) were the same (Figure 4). Temperature had a significant effect on seed germination. This represents the tolerance of the plant in the different temperature ranges. This is because the mentioned plant in the Zagros Mountain, able to adapt to different temperatures and with germination in a relatively wide temperature range, guarantees its survival. Light and temperature are two environmental factors that impact on multiple levels of germination. The highest germination rate was observed in the treatment combination of 15/25 (day/night),

0mM of NaCl, 1cm of planting depth and pH=7. Alternating temperatures increase germination of seeds (Martinez-Ghersa et al., 2003).

Table 3. Analysis of mean of variances for effects of salinity, temperature, pH and sowing depth on germination characters of *Thymus daenensis* and *T. vulgaris* in the first step.

Source	d.f	<i>Thymus daenensis</i>			<i>T. vulgaris</i>		
		G.R	S.V	G.P	G.R	S.V	G.P
Salinity (A)	6	12.2**	35.5**	64.4**	14.5**	48.98**	55.32**
Temperature (B)	4	8.8**	24.3**	42.2**	9.14**	35.78**	27.1**
pH (C)	4	7.2**	23.3**	63.3**	14.5**	37.98**	29.89**
Sowing depth (D)	3	15.6**	47.7**	77.8**	14.9**	55.98**	114.2**
A× B	24	1.5*	3.95*	18.76**	1.37*	5.03*	24.5**
A× C	24	1.54*	4.01*	6.62*	1.45*	4.99*	45.76**
A× D	18	1.52*	4.19*	7.02*	1.41*	5.63*	117.8**
B× C	16	2.7**	4.25*	7.12*	4.51**	5.71*	8.44*
B× D	12	3.5**	4.56*	7.63*	6.97**	6.15*	9.2*
C× D	12	4.4**	5.01*	7.55*	12.7**	6.18*	9.43*
A× B× C	96	0.94*	2.58*	4.32*	0.89*	3.48*	5.14*
A× B× D	72	1.14*	3.16*	5.29*	1.06*	4.26*	6.31*
A× C× D	72	1.16*	3.2*	5.15*	1.02*	4.29*	6.37*
B× C× D	48	1.265*	3.49*	5.85*	1.14*	4.71*	6.99*
A× B× C× D	288	0.0045 <sup>ns</sup>	0.0005 <sup>ns</sup>	0.024*	0.00056 <sup>ns</sup>	0.021*	0.03*
Error	210	1.1	3.04	5.09	0.99	4.08	6.03
C.V		11.1	14.5	8.7	7.4	6.5	5.9

Maximum germination in seeds was performed at red light and alternating temperatures (Tang et al., 2008). Annual seeds sprout in late spring or early summer to complete their life cycle. Seeds of plants must be exposed to high temperatures in the summer months (Baskin and Baskin, 1998). In *Phalaris arundinacea* better germination was observed in 16 hours of lighting (Lindig-Cisneros and Zedler, 2001), but in another study it has been reported that the best temperature was 20° C as well as light regime of 12 h in dark conditions (Kon et al., 2007). The germination and maturity responses to latitude, elevation, soil moisture, soil nutrients, temperature and vegetation density of habitat destruction are different (Baskin and Baskin, 1998). Germination and seedling growth can be reduced by some non-living factors such as salinity and drought, which are the

most important abiotic stresses to limit the number of seedlings and seedling growth (Atak et al., 2006; Kaya et al., 2006). Salinity is an important factor in the peripheral condition that threatens the sustainability of arid and semiarid regions, especially in areas where evapotranspiration is greater than precipitation (Szabolcs, 1994). High salinity usually decreases the rate and extent of germination. Salinity inhibits germination of seeds by reduced water availability or interferes with some aspects of metabolism like changing the balance of growth regulators. In canary grass, the highest percentage of germination (96.5 %) in the control treatment was observed and in salinity of 320 mM sodium chloride, germination was stopped (Ahmadi et al., 2013). In this research, fresh weight and dry mass yield of plants slightly decreased as the salinity increased. The highest tolerance to salinity in the seeds of the medicinal herbs of *Langematia iberica*, *Plantago major*, *Anethum graveolens*, *Cuminum cyminum*, *Trifolium subterraneum*, *Trachyspermum ammi*, *Origanum majorana* L., *Lactuca sativa*, *Sesamum indicum*, *Trigonella foenum*, *Alyssum desertorum*, and *Portulaca oleracea* L. was in *Portulaca oleracea* L., *Alyssum desertorum*, and *Trigonella foenum*, and it was up to 450 mM/l while the lowest tolerance was reported in *Plantago major*, *Langematia iberica*, and *Anethum graveolens* (Yadegari, 2015b). In a similar study on *Linum usitatissimum* L., *Echinacea angustifolia*, *Carthamus tinctorius* L., and *Cynara scolymus* L., the highest salinity stress tolerance was observed in the seeds of *Linum usitatissimum* L. and *Carthamus tinctorius* L. at the germination stage (Gholizadeh et al., 2016). A response to several salinity concentrations depends on many reasons, for example species of plant and osmotic regulation. Yield and biomass reductions are very common under salt stress conditions, especially for salt-sensitive crops, due to osmotic effects and ionic imbalances (Bannayan et al., 2008; Lattanzio et al., 2009; Myung et al., 2009). Ahmadi et al. (2013) found similar results in a study on germination of *Phalaris minor*. Percentage and germination rates of seeds of canary grass were influenced by acidity (Ahmadi et al., 2013). The most important effect of pH is the availability of nutrients in soil. The elements such as calcium, phosphorus and potassium are leached or insoluble in low pH, and on the other hand, the elements such as iron, manganese and other micro-nutrients are unavailable in high pH (Yadegari, 2017a, b). Most studies about the effect of depth on seedling emergence of plants showed that by increasing of sowing depth, emergence of seedlings reduced (Benvenuti, 2003; Mohler, 2001). Yield and biomass reductions are very common under salt stress conditions, especially for salt-sensitive crops, due to osmotic effects and ionic imbalances (Attia et al., 2011). Biological reasons for the lack of germination have not been specified yet completely. The seedling emergence in different depths depends on the seed energy reserves (Ren et al., 2002).

Table 4. Analysis of mean of variances for effects of salinity, temperature, pH and sowing depth on germination characters of *Thymus daenensis* and *T. vulgaris* in the second step.

Source	d.f	<i>Thymus daenensis</i>			<i>T. vulgaris</i>		
		G.R	S.V	G.P	G.R	S.V	G.P
Salinity (A)	6	42.2**	57.7**	89.9**	55.87**	77.93**	77.19**
Temperature (B)	4	12.8**	44.3**	66.8**	89.54**	65.98**	34.65**
pH (C)	4	14.2**	88.5**	89.86**	44.35**	87.65**	44.73**
Sowing depth (D)	3	19.6**	66.7**	82.76**	33.65**	63.78**	69.77**
A×B	24	15.5**	14.78**	45.65**	5.44*	6.78*	64.78**
A×C	24	14.78**	6.6*	9.43*	5.51*	6.81*	77.94**
A×D	18	1.62*	7.05*	9.95*	5.82*	7.22*	93.75**
B×C	16	5.5**	7.15*	10.22*	5.92**	7.33*	11.84*
B×D	12	7.7**	7.62*	10.85*	6.35*	7.89*	12.95*
C×D	12	12.4**	7.65*	11.16*	44.66**	7.85*	12.94*
A×B×C	96	0.99*	4.38*	6.17*	3.63*	4.45*	6.98*
A×B×D	72	1.23*	5.36*	7.55*	4.41*	5.51*	8.56*
A×C×D	72	5.8**	5.42*	8.05*	4.47*	5.58*	8.51*
B×C×D	48	1.33*	5.92*	8.31*	4.77*	6.021*	9.34*
A×B×C×D	288	0.0009 <sup>ns</sup>	0.000075 <sup>ns</sup>	0.00024 <sup>ns</sup>	0.0000081 <sup>ns</sup>	0.026*	0.042*
Error	2100	1.21	5.08	7.17	4.156	5.19	8.16
C.V		9.93	8.18	7.76	9.14	5.8	4.42

ns: Not significant, \* and \*\* : Significant at  $P \leq 0.05$  and  $P \leq 0.01$  levels respectively. (G.P: germination percentage, G.R: germination rate, S.V: seed vigour).

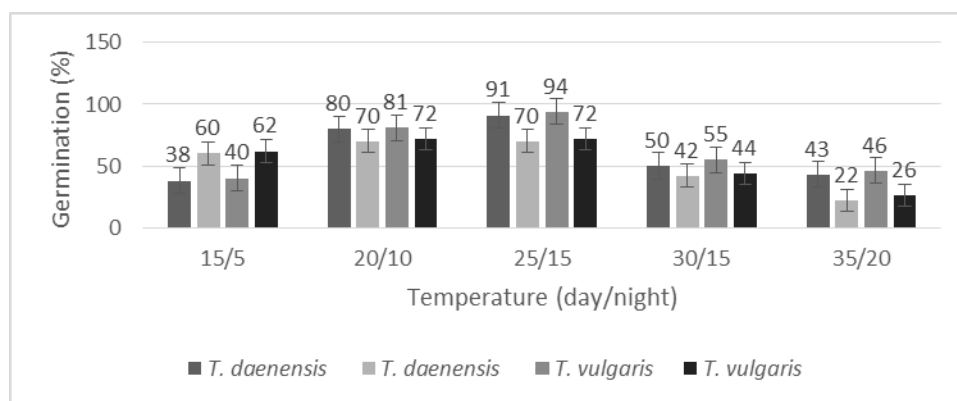


Figure 1. The effect of alternating temperatures (day/night) on *T. daenensis* and *T. vulgaris* seed germination.

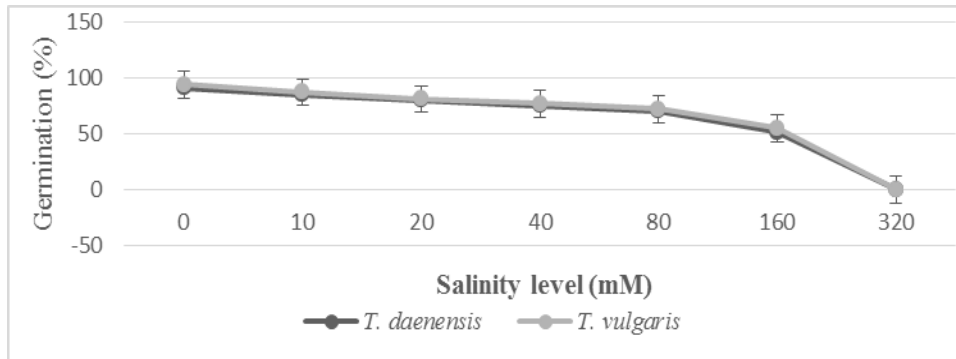


Figure 2. Final germination percentage of *T. daenensis* and *T. vulgaris* under different salt stresses.

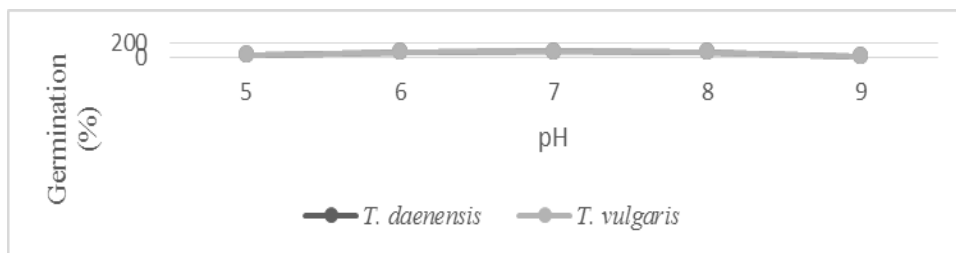


Figure 3. Germination percentage of *T. daenensis* and *T. vulgaris* under various pHs and temperatures 15°/25° C (day/night).

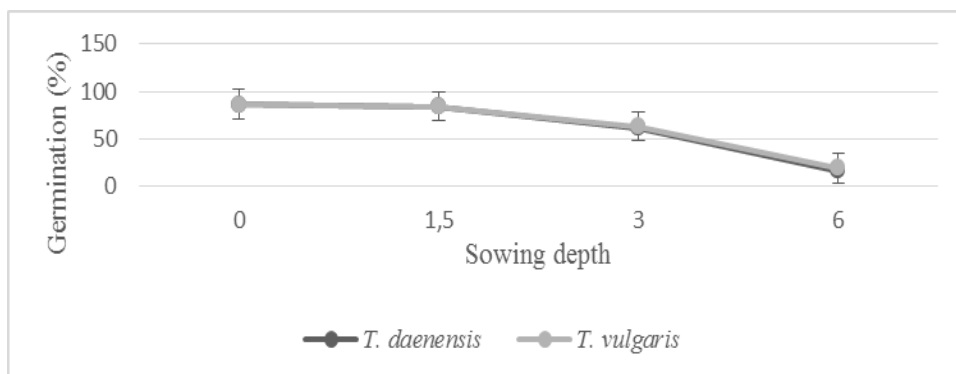


Figure 4. Germination percentage of *T. daenensis* and *T. vulgaris* under various sowing depths and temperatures 15°/25°C (day/night).



A high concentration of NaCl in lettuce in nutrient solution strongly affected the germination rate and root elongation, seedling and mature vegetative growth of both sesame and lettuce (Myung et al., 2009). By increasing of salinity levels, seedling emergence of medicinal plants decreased. Responses to several salinity concentrations depended on many reasons. In addition, seed germination and seedling emergence are influenced by moisture availability, temperature and light levels (Chauhan and Johnson, 2008). Enhanced radiation decreased plant height, dry weight of individual stem and yield per plant, plant growth and development, photosynthesis and biomass production (Liu et al., 2013; Choudhary and Agrawal, 2014). With the increase in levels of drought stress by increasing of sowing depth, seed accessing to water was reduced. In this way, it is possible that the germination percentage will be reduced (Ansari et al., 2012). Generally, with the increase of drought stress, the ability of suction of water by seeds will be decreased and the necessary duration for water sucking will be increased and consequently the start of germination processes will be postponed (Ghaderi et al., 2010). Destructive effects of salinity levels and a decrease of growth parameters in *Thymus broussonetii* Boiss (Belaqziz and Romane, 2014), *Nigella sativa* (Bourgou et al., 2012), *Suaeda maritime* (Gazala et al., 2013), *Artemisia annua* L. (Irfan Qureshi et al., 2013), *Schinopsis quebracho* (Meloni et al., 2008), *Carthamus tinctorius* L. (Salem et al., 2014) and *Capsicum* (Patade et al., 2011) were previously reported.

### Conclusion

Treatments with superior levels of salinity, pH, sowing depth and temperature had more negative effects on germination characters than other treatments. Emergence decreased with an increased concentration of salinity, planting depth and pH. In two species, the greatest germination percentage was made by combination of treatment of 15/25 (day/night), 0mM of NaCl, 1cm of planting depth and pH=7. The least germination percentage was produced by the combination of treatments of 35/20 (day/night), 320mM of NaCl, 6cm of planting depth and pH=9. This study provides some useful information about the efficacy of environmental effects (salinity, temperature, pH and sowing depth) on germination characters of two species of thyme. These methods are relatively new and need further improvement with regard to rates, timing, and techniques.

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UTICAJ FAKTORA ŽIVOTNE SREDINE NA KARAKTERISTIKE  
KLIJAVOSTI SEMENA *THYMUS DAENENSIS* I *T. VULGARIS*

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R e z i m e

Ovo istraživanje je sprovedeno radi ispitivanja uticaja saliniteta, temperature, pH i dubine setve na karakteristike klijavosti dve vrste majčine dušice 2017. godine. Tretmani su sadržavali: a) promenljive temperature u klijalištu 5/15, 10/20, 15/25, 15/30 i 20/35 0C (dan/noć), b) različite koncentracije rastvora soli 0, 10, 20, 40, 80, 160 i 320 mM NaCl, c) dubinu setve od 0, 1,5, 3 i 6 cm, pH vrednosti od 5, 6, 7, 8 i 9. Rezultati su pokazali da su efekti različitih tretmana bili značajni na procenat klijavosti, stopu klijavosti i vigor semena. U većini slučajeva, najveće koncentracije svih tretmana su imale negativan efekat na karakteristike klijavosti. Klijavost se smanjivala sa povećanom koncentracijom soli, dubinom setve i pH vrednosti. Kod obe vrste, najveći procenat klijavosti (94%) postignut je kombinovanim tretmanom 15/25<sup>0</sup>C (dan/noć), 0 mM NaCl, 1 cm dubine setve i pH=7. Klijanje je zaustavljeno u kombinaciji tretmana 35/20 (dan/noć), 320 mM NaCl, 6 cm dubine setve i pH=9. Pomenuta kombinacija tretmana imala je jači inhibitory i destruktivni efekat od pojedinačnih tretmana.

**Ključne reči:** pH, salinitet, dubina setve, temperatura, majčina dušica.

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