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Germination and Emergence Ecology of *Thymus vulgaris* and *Thymus daenensis*

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ABSTRACT

Understanding the germination of medicinal plants to develop long-term strategies of their management can be useful. In sowing these plants, due to the high sensitivity of their seed germination, it needs to the comprehensive information on how to grow and make desirable seedbed to germination. In order to understand some ecological characteristics of *Thymus vulgaris* and *T. daenensis*, Factorial experiments carried out in a Randomized complete-block design with four replications at Islamic Azad University of Shahrekord in 2015. Effect of temperature on seed germination were evaluated in germinator under night/day fluctuating temperatures (5/15, 10/20, 15/25, 15/30 and 20/35° C), effect of salinity by using NaCl solutions (0, 10, 20, 40, 80, 160 and 320 mM) and acidity effect by using buffer solutions with adjusted pH 5 to 9. To study the effect of seed planting depth on seedling emergence, seeds were buried at different depths (0, 1, 2, 3 and 4 cm). Results showed that the effect of different temperatures on percentage, speed and time to reach 50% of maximum germination in relation to the both varieties were not significant. Germination percentage of *T. vulgaris* related to alternating temperatures 15/25° C night/day was by 96% during the day and the lowest germination belonged to 20/35° C by 37% and 0% respectively during the day and night. The highest germination percentage of *T. daenensis* was in 20/35° C by 93% in day, but other temperatures of seed germination belong to this plant was relatively acceptable. Increasing salinity reduced germination of both species. General emergence decreased with increasing planting depth. Acidity treatments had significant effect on germination. Minimum and maximum germination of *T. vulgaris* were observed at pH 9 and 7 up to 20% and 93%, respectively. The highest and lowest germination percent of *T. daenensis* were at pH 7 and 9 up to 94% and 19%, respectively.

1. Introduction

Understanding the biology of the plant germination and grow crops are undeniable necessity in effective management systems. Seed dormancy is a complex process which plant establishes seedling coincided with favorable conditions. Most crops have been domesticated from wild species and show decreased levels of seed dormancy compared with their wild relatives, which ensures higher emergence rates after sowing (Lenser and Theissen, 2013 ; Meyer and Purugganan, 2013). However, the inappropriate loss or release of seed dormancy results in the rapid germination of freshly matured seeds or even pre-harvest sprouting (vivipary) in crops, causing substantial losses in yield and quality in agricultural production in addition to problems including post-harvest management and subsequent subsequent in-

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dustrial utilization (Simsek et al., 2014). Affecting Factors on germination and emergence are temperature, osmotic pressure of the solution, the quality of light, the position of the seed in the soil seed bank and soil texture. Seed germination and seed vigor can be significantly influenced by changes of after ripening time and temperature (Qu and Huang, 2005). Seed germination and emergence depend to depth in the soil. The depths more than of optimum reduce the emergence significantly. Effects of acidity on germination potential vary in different plants. Some species are more acidic conditions some prefer Alkaline or neutral pH some others do not show any reaction (Susko et al., 1999; Pierce 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). However, generally germination rate of plant species, like Canary grass, is very low due to seed dormancy (Radosevich et al., 1997). Overall germination is an adjusted event by several environmental factors such as temperature, light, salinity, pH and moisture and it is key to the success of plants in agro ecosystems (Chauhan et al., 2006). One of the main problems in the cultivation of medicinal plants is non-well established in field conditions. Sowing seeds of medicinal plants due to the high sensitivity which these plants seeds show to grow and develop, need full information on how prepare seed germination bed. In this regard, insufficient information is available to experts. Wild population's seeds of medicinal plants that have been growing in different environments have shown different levels of sleep. *T. daenensis* is an herb endemic to Iran belonging to the Lamiaceae family (Rechinger, 1982). Growing in many parts of Iran, the plant is extensively used in folk medicine. Mentioned in the all of the pharmacopoeia of drugs known as thyme and its benefits have been emphasized on health. Thyme morphological diversity can affect ecological factors of plant competition, herbivores, time of germination, flowering time and genetic effects (Corticchiato et al., 1998). Hence, the aim of this study was the understanding ecological characteristics of *T. daenensis* and *T. vulgaris* in terms of seeds germination.

2. Materials and Methods

The investigation was conducted in Laboratory, Faculty of Agriculture Islamic Azad University of Shahrekord at 2015. Used seeds were collected at the mountains of Chahar Mahale-Bakhtiari.

2.1. Germination

The experiment was conducted in four replications; each of them is consisting of 25 seeds *T. daenensis* and *T. vulgaris* for each treatment in which seeds germinated at 20°C. Seeds had placed on two moistened paper towels. After covering the seeds with a third sheet of paper, the three towels had loosely rolled to form a tube and placed in plastic bags (23 × 33 cm) to prevent evaporation. Seeds were observed twice daily and considered germination when the radicle was approximately 2 mm long. Estimates of time taken for cumulative germination to reach 50% of its maximum at each replicate (D50) were interpolated from the germination progress curve versus time. Germination rate (R50 1/h) was then calculated according to following formula (Soltani et al., 2001): R50 = 1/D50

2.2. Temperature and Light

Separate experiment conducted to find the optimum temperature and light requirement for seed germination in both *T. daenensis* and *T. vulgaris*. Seed germination was determined in growth chambers under fluctuating day/night temperatures (5/15, 10/20, 15/25 and 20/35°C). These temperature regimes were selected to reflect temperature variation during the spring to summer period in Iran.

2.3. Salt and Osmotic Stress

Seed germination as influenced by salt stress was evaluated using sodium chloride (NaCl) solutions of 0, 10, 20, 40, 80, 160, and 320 mM. To evaluate the potential salinity on seed germination reduction, the three-parameter logistic model was used:

$$Y = a/[1+(X/X50)\ b]$$

Y is germination in salinity level of X (%); a is maximum germination (%); X50 is salinity level required for 50% inhibition of maximum germination, and b is slope represents of reduced germination by increasing salinity (Chauhan et al., 2006).

2.4. Acidity of Buffered Solution

Acidity effect on seed germination was evaluated using buffer solutions of pH 5 to 9 prepared according to the method described by Chachalis and Reddy, 2000. A 2-mM buffered solution of potassium hydrogen phthalate was adjusted to pH 4 with 1 N HCl. A 2mM solution of MES [2-(N-morpho-lino) ethane sulfonic acid] was adjusted to pH 5 or 6 with N HCl or NaOH. A 2mM solution of HEPES [N-(2-hydroxymethyl) piperazine-N'-(2-ethanesulfonic acid)] was adjusted to pH 7 or 8 with 1 N NaOH. A pH 9 or 10 buffer was prepared with 2mM tricine [Tris (hydroxyl methyl) methyl glycine] and adjusted with N NaOH. Non-buffered deionized water (pH 6.3) was used as control.

2.5. Burial Depth

The effect of seed burial depths on *T. daenensis* and *T. vulgaris* seedling emergence was studied in a greenhouse. Fifty seed from each one were buried in soil in 15-cm-diam plastic pots at depths of 0, 1, 2, 3, and 4 cm. Soil used for this experiment was a clay loam with 3.1% organic matter. Pots in which not buried plants seed were set up to determine the background *T. daenensis* seed bank. There was no emergence of cheat grass from these control pots during the course of the experiment, suggesting that there was no background seed bank of Thymus in the soil. The temperature of the glasshouse was set at 25/15-5 °C (day/night). Pots were watered initially with an overhead sprinkler and later with sub irrigation as needed. Emergence defined as the appearance of two cotyledons, and the experiment was done until 30 days after burial.

2.6. Statistical Analyses

Factorial experiments in a randomized complete block design with four replications were applied in all experiments. Each replication was arranged on a different shelf within the germination chambers and considered as a block. Each treatment was conducted twice except the burial depth. Statistical analysis has done by using SAS software to test and compare the based on FLSD (LSD protected) at the 5% probability level. Germination rate and time to reach 50% of maximum germination have calculated by GERMIN program in Excel software. GERMIN PROGRAM that works in Excel and germination data for analysis in other statistical software, its standardization.

3. Results and Discussion

3.1. Temperature and Light

In *T. daenensis*, the highest percentage of germination was in 15/25°C (day/night) treatment by 93% (Fig. 1a). However, at the other temperatures, seed germination for this medical plant was relatively acceptable. In addition, this represents the tolerance of the plant in the different temperature ranges. For this reason, the mentioned plant in the Zagros Mountain is able to adapt to different lightness and with germination in a relatively wide temperature range guarantee its survival. In *T. vulgaris*, light effect was significant on all measured parameters. Effect of temperature on rate of germination and time to reach 50% of maximum germination were not significant, but its effect on the rest of the other parameters was significant (Table 1). Nevertheless, this study showed that rate of germination (R50) has not been affected by temperature, but more investigations in this field can reveal better results. As it is observed in Fig. 1b, the highest percentage of germination was related to alternative temperatures 15/25°C by 96% in day and 10/20°C by 90% and its lowest was belonged to 20/35°C by 37 during day and zero percent at the night. It indicates that temperate conditions are desirable to germination of *T. vulgaris*. Light and temperature are two environmental factors that affect multiple levels of dormancy. It is said that alternating temperatures to break dormancy seeds of barnyard grass, common Lamb's quarters and Red root pigweed increased dramatically (Martinez-Ghersa et al., 2003). It is reported that maximum Chenopodium germination in seed placement was at red light and alternating temperatures (Tang et al., 2008). According to the above results, a few points are clear. Only a few of these species have a secondary dormancy mechanism that will prevent germination in the spring (Baskin and Baskin, 1983) including winter races and many winter annual plant that can exert phenology of plants that found in Spring crops (Hald, 1999). Second maybe compared with species that may sprout in late spring, a little overlap in species that germinate in early spring bud. By examining the light on *Phalaris arundinacea* is indicated better germinate-

Table 1. Effect of light conditions and temperature regimes on percentage, speed and time to reach 50% of maximum germination in all parameters of *T. daenensis* and *T. vulgaris*. (Gmax: maximum germination, R50: minimum until germination G°, GU: uniform germination, D50: takes a while to reach its maximum of 50% germination).

Thymus	Source of Variation	Mean Square							
		Gmax	R50	GU	D05	D10	D50	D90	D95
T. daenensis	Rep	53.78 ns	665.45 ns	687.54 ns	21.89 ns	60.87 ns	319.54 ns	414.76 ns	365.98 ns
	Light (L)	1323.76 **	679.87 ns	3421.87 **	234.87 **	845.87 **	324.87 ns	543.87 ns	234.98 ns
	Temperature (T)	7564.83 **	2395.43 *	4365.65 **	324.72 **	759.76 **	148.98 ns	211.09 *	391.79 ns
	L*T	432.87 **	546.81 ns	3987.45 **	349.76 **	119.54 ns	89.67 ns	745.89 ns	804.56 ns
	C.V	14.3	7.63	15.61	6.76	6.42	7.83	8.44	8.52
T. vulgaris	Rep	49.74 ns	564.984 ns	657.94 ns	19.956 ns	59.93 ns	321.73 ns	412.98 ns	345.89 ns
	Light	1429.63 **	4376.76 **	3498.58 **	245.76 **	987.95 **	2084.94 **	4324.56 *	5445.78 *
	Temperature	7693.93 **	765.48 ns	4435.98 **	434.76 **	867.94 **	132.78 ns	5245.3 **	7698.45 **
	L*T	546.83 **	698.65 ns	400.1.45 **	468.912 **	797.87 **	543.6 *	210.45 *	212.83 *
	C.V	13.43	8.76	14.32	7.23	6.45	7.87	8.83	9.12

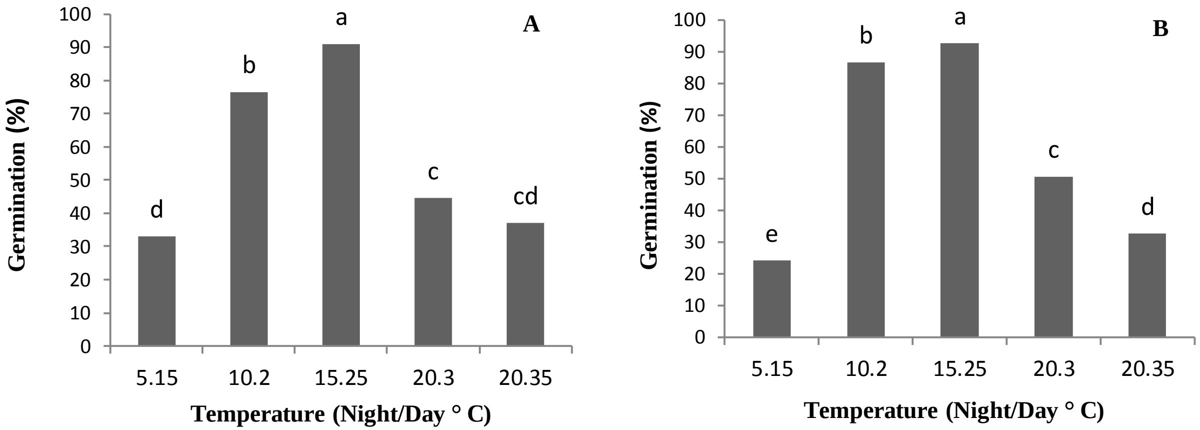

Fig. 1. Effect of alternating temperatures on *T. daenensis* (A) and *T. vulgaris* (B) seed germination. Column without a common letter significantly different at 5% level are based on FLSD test.

on rate by 80% in white light for 16 hours (Lindig-Cisneros and Zedler, 2001). In another study, *Phalaris arundinacea* at 20 ° C and 12 h light regime levels showed by 88% germination (Kon et al., 2007). Depending on the species, germination response with latitude, elevation, soil moisture, soil nutrients, temperature, type and concentration, vegetation density degree of habitat destruction, the seeds are mature differently in places (Baskin and Baskin, 1998).

3.2. Salinity

In *T. daenensis*, salinity was significant on all measured parameters. The fitted model was significant ($P<0.001$, $r2= 0.98$) (Fig. 3a). The highest percentage of germination was observed in 20 mM treatment by 91% and it was stopped in salinity by 320 mM sodium chloride germination. By 50% reduction in germination occurred at salinity 165.90 mM sodium chloride (Fig. 2a). In *T. vulgaris*, increasing salinity reduced germination percentage (Table 2). The highest germination percentage was observed in control by 97.5 and in salinity with 240 mM sodium chloride germination was stopped that it was the lowest (Fig. 2b). In addition, the fitted model was significant ($P<0.001$, $r2 = 0.98$) (Fig. 3b). Increasing salinity affected the rest of the measured parameters significantly (Table 2). Germination and seedling growth can be reduced by some non-living factors such as salinity and drought that are the most important abiotic stresses to limit the number of seedlings and seedling growth (Almansouri et al, 2001; Atak et al., 2006; kaya et al., 2006). Salinity is an important factor in the peripheral that threaten the sustainability of arid and semiarid regions, especially in areas where evapotranspiration is greater than precipitation (Szabolcs, 1994). High salinity usually reduces the rate and extent of germination. Salinity inhibits seeds germination through decline in water availability or interferes with some aspects of metabolism like changing the balance of growth regulators. It is considered that the highest percentage of canary grass germination in control treatment was observed about 96.5% and germination was stopped in salinity 320 mM sodium chloride (Ahmadi et al., 2013). Decline of 50% germination occurred at salinity 40 mM sodium chloride.

3.3. Acidity

In *T. daenensis*, acidity affected rate and percentage of seed germination. Maximum germination achieved at pH 7 by 94% and lowest at pH 9 by 19%, respectively (Fig. 4a). Quadratic model fitted well to the germination changes of justified acidity ($P<0.05$, $r2=0.96$). In *T. vulgaris*, the effect of acidity on germination percentage was significant (Table 2) and the fitted model justified it well ($R2 = 0.99$). The highest and lowest percentage of germination was seen at pH 7 and 9, respectively by 93% and 20% (Fig. 4b). Ahmadi et al. 2013 found similar results in a study of germination *Phalaris minor*. They also observed that acidity affected percentage and germination rate of Canary grass seeds. Maximum germination achieved at pH 7 by 96% and lowest at pH 5 by 30%, respectively. The most important effect of pH is on the availability of nutrients that can be affected in soil. At the very low pH, elements such as calcium, phosphorus and potassium are leached from the soil or they are converted to insoluble form that plants cannot absorb them. In high pH, soil may be deficient in terms of phosphorus, iron, manganese and other micronutrients (Seeber, 1976).

3.4. Burial Depth

In *T. daenensis*, planting depth affected seedling emergence at soil surface (zero depth) was maximum (86.35%), and in 1.5 cm cultivation depth was by 85.77% (Fig. 5a). The model was fitted to this trend, could well justify the emergence with increasing the depth ($P<05$, $r2= 0.95$) and this indicates that with 3.63 cm planting depth, 50% reduction occurred in emergence of *T. daenensis*. In *T. vulgaris*, with increasing sowing depth, seedling emergence decreased significantly (Table 2), and fitted model has properly showed the tendency ($P<05$, $R2= 0.94$). Percentage of emergence by 82.25 and 88.57 percent were observed respectively at the surface of soil and a depth of 1.5 cm and at soil depth of 4.5 cm was recorded by 8.35 percent. Based on the fitted model, the depth that cause to emergence above 50% of seedling was determined by 3.39 cm (Fig. 5b). Most studies about the depth effect on seedling emergence have shown that with increasing burial depth, seedling emergence decrease in an exponential trend (Cussans et al., 1996; Benvenuti, 2003; Mohler, 2001; Grundy et al., 1996; Ahmadi et al., 2013).

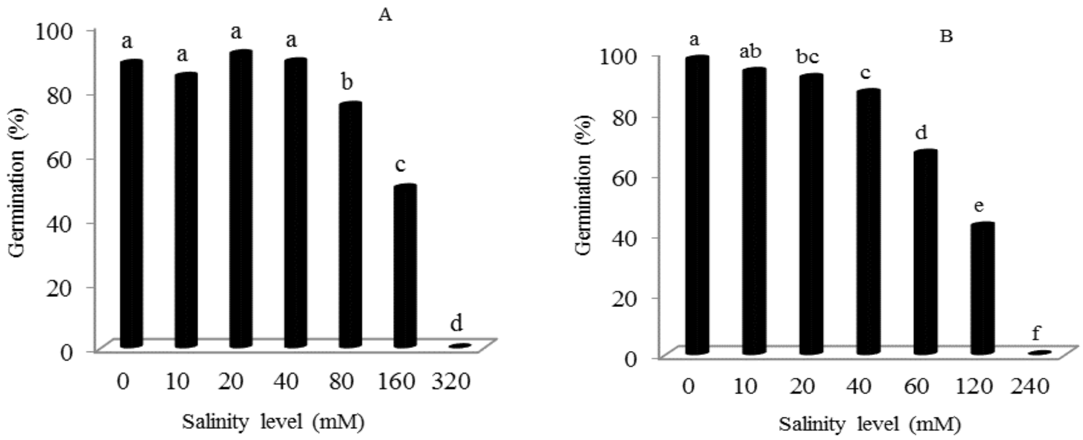

Fig. 2. Effect of salinity levels on *T. daenensis* (A) and *T. vulgaris* (B) seed germination. Column without a common letter significantly different at 5% level are based on FLSD test.

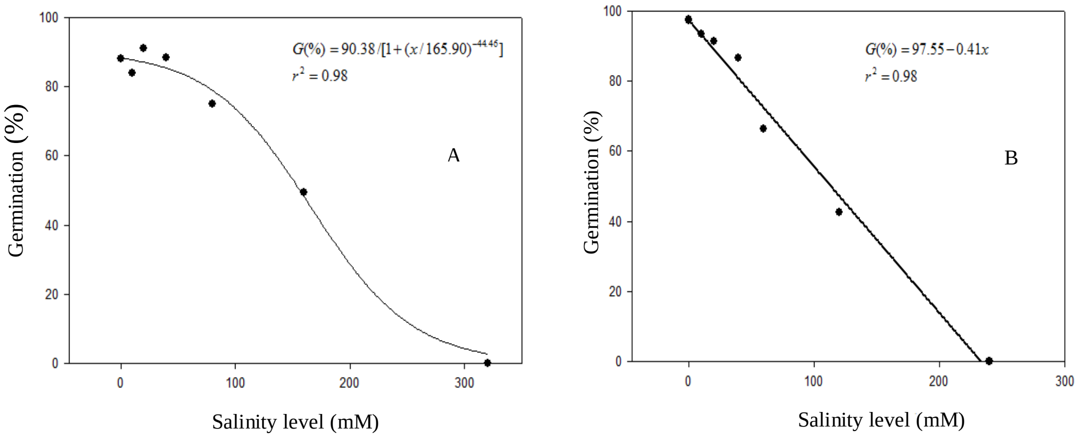

Fig. 3. Percentage of final germination potential in *T. daenensis* (A) and *T. vulgaris* (B) under different stress salt from sodium chloride (NaCl). Points represent observed data and lines that are fitted with the logistic equation.

Biological reasons for the lack of germination, deep down, not specified yet completely. However, it is certain that the emergence from different depths is proportional related to the seed energy reserves (Lafond and Baker, 1986). In addition, burial depth can affect in seed germination and seedling emergence through the moisture availability, temperature and light (Chauhan and Johnson, 2008). By studying effect of humidity and the soil depth on the emergence of *B. tectorum* was observed that the highest amount of this herb emergence happened and with increasing depth of soil, the emergence of *B. tectorum* has been reduced (Shahrokhi et al., 2011). According to observations by other researchers when seed on desired depth emergence undergo an exponential decrease in seedling emergence to occur (Nazarialam et al., 2012; Lafond and Baker, 1986).

4. Conclusion

Seeds germinated to higher percentages in light than in darkness and at high than at low temperatures. *T. vulgaris* and *T. daenensis* emerged best when the temperature ranged between 20°C and 25°C, indicating their preference for cool climates or cool periods for seedling emergence during the year in areas where the climate is hot. The optimal range of acidity to germination and emergence of the both Thymus was by 5.5-7.5 dSm-1. On the other hand,

Table 2. Effect of salinity, pH buffer solution and sowing depth on all parameters measured in the *T. daenensis* and *T. vulgaris*. (Gmax: maximum germination; R50: minimum until germination G°; GU: uniform germination; D50: takes a while to reach its maximum of 50% germination).

Thymus	Test	Source of Variation	df	Mean Square							
				Gmax	R50	GU	D05	D10	D50	D90	D95
T. daenensis	Salinity	Rep	6	18.7 ns	4.56 ns	1256.8 ns	14.87 ns	94.76 ns	276.87 ns	768.9 ns	987.65 ns
		Treat	3	4003.5 **	48.673 **	2543.8 **	354.87 **	3432.54 **	834.8 **	5432.54 **	6576.61 **
		CV		11.54	10.23	14.76	7.12	12	8.39	9.34	10.34
	Acidity	Rep	4	13.4 ns	0.000056 ns	7.65 ns	24.67 ns	27.54 ns	51.86 ns	327.76 ns	153.67 ns
		Treat	3	4153.6 **	0.000002 ns	654.87 ns	31.67 ns	35.54 ns	65.76 ns	409.29 ns	169.58 ns
		CV		8.34	21.23	20.20	25.23	24.34	15.23	11.34	10.54
	Sowing Depth	Rep	5	1.76 ns	-	-	-	-	-	-	-
		Treat	3	5865.76 **	-	-	-	-	-	-	-
		CV		8.34	-	-	-	-	-	-	-
T. vulgaris	Salinity	Rep	6	16.76 ns	3.56 ns	1198.53 ns	13.42 ns	88.35 ns	198.73 ns	745.83 ns	879.36 ns
		Treat	3	4231.87 **	56.53 **	2763.87 **	453.81 **	3543.94 **	901.62 **	5543.27 **	6783.92 **
		CV		9.44	21.15	20.12	25.34	24.56	15.78	12.45	11.38
	Acidity	Rep	4	12.87 ns	0.000032	6.54 ns	23.89 ns	635.98 *	50.53 ns	323.983 ns	152.98 ns
		Treat	3	34.65 ns	0.0000192	342.73 ns	29.987 ns	34.73 ns	62.37 ns	408.219 ns	165.24 ns
		CV		10.26	15.34	16.76	23.23	26.45	17.45	14.00	13.34
	Sowing Depth	Rep	5	2.356 ns	-	-	-	-	-	-	-
		Treat	3	5982.65 **	-	-	-	-	-	-	-
		CV		2.05	-	-	-	-	-	-	-

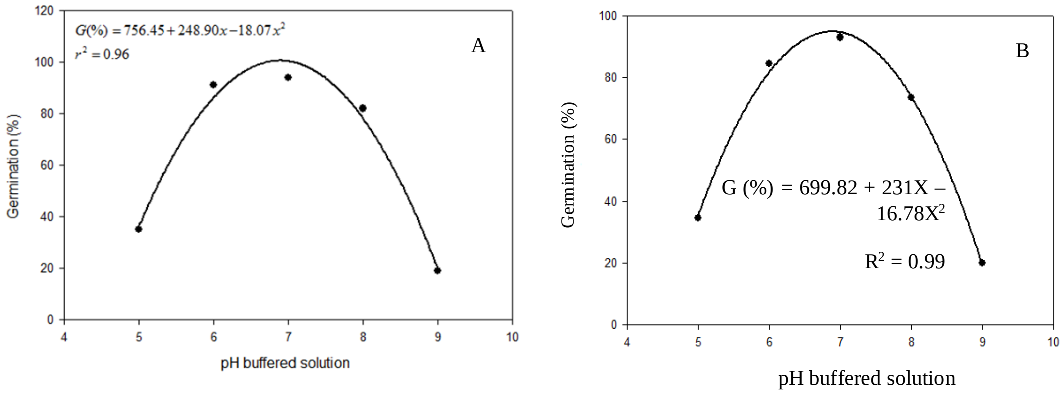

Fig. 4. Effect of buffer solution pH *T. daenensis* (A) and *T. vulgaris* (B) germination temperature 15/25°C (day/night) with 12 h light period. Model fitted quadratic model to represent the data.

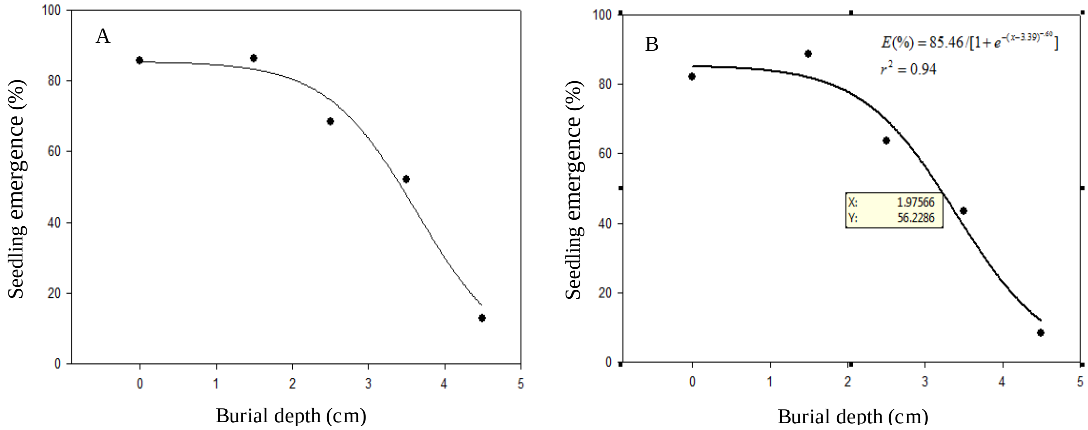

Fig. 5. The effect of burial depth on seedling emergence of *T. daenensis* (left or right) and *T. vulgaris* (left or right), temperature 15/25°C (day/night) with 12 h light period of 30 days after planting. Represents the fitted sigmoidal model for data reduction.

the lower or higher pH will prevent germination and emergence. Probably, too acidic and basic conditions inhibit the enzymes activities and lead to proteins denaturation. Obviously, the results suggest that salinity reduces seed germination and emergence as well as. With increasing salinity from 100 mM NaCl, germination percentages decreased. Salinity through the raising of osmotic pressure leads to the decline of germination percentage and boost average germination time. Salinity disturbs the metabolic and physiological processes during plant growth especially in germination period. The study showed that with increasing burial depth from 1.5 cm, germination and emergence is reduced. At greater depths, the induction of secondary dormancy is the main reason for the reduction of germination in the seeds. With increasing depth of planting, seed dormancy was due to hardening of the gas exchanges. Moreover, low emergence with increasing depth of seed may be related to the energy stored in the seed. However, germination and emergence are functions that are affected by several environmental factors.

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