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Effects of Temperature and Salinity on Germination of Milk Thistle (*Silybum marianum*)

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Abstract: This laboratory study evaluated how temperature and salinity affect seed germination. *Silybum marianum* seeds were subjected to incubation temperatures of 10, 15 and 20°C with varying levels of saline water during germination. The saline levels were 1, 3, 5, 7 and 9 deciSiemens per meter (dS m^{-1}). *Silybum marianum* seeds also were tested using standard germination protocols, with the salt content of the wetting solution remaining constant. Germination was evaluated 6, 9, 12, 15, 18 and 21 days after placement in the incubators. Seeds incubated at 10°C did not germinate. At an incubation temperature of 15°C, less than optimal germination occurred during the 21 day test. After only 12 days, *Silybum marianum* seeds tested at 20°C came to within 90% of maximum germination. Salinity levels at or above 7 dS m^{-1} reduced germination by as much as 19% under 20°C conditions and by as much as 27% under 15°C conditions. Extrapolating these results to field conditions suggests that *Silybum marianum* should be planted into a seedbed with salinity less than 3 dS m^{-1} and an average temperature of 20°C for 12 days.

Key words: Temperature, salinity, germination, *Silybum marianum*

INTRODUCTION

Silybum marianum (L.) Gaertner., Milk thistle, extracts were used as early as the 4th century B.C., became a favored medicine for hepatobiliary diseases in the 16th century and have been used in Europe during this century (Burgess, 2003; Schuppan *et al*., 1999). Milk thistle grows natively in the Mediterranean (Morazzoni and Bombardelli, 1995; Murphy *et al*., 2000) and is widespread in other regions in the world including Iran (Shokrpour *et al*., 2007). It is currently purported to have value as a liver protectant to lessen damage from potentially hepatotoxic drugs and for treating liver disorders including toxic liver damage caused by chemicals, Amanita phalloides mushroom poisoning, jaundice, chronic inflammatory liver disease, hepatic cirrhosis and chronic hepatitis (Dvorak *et al*., 2003; Saller *et al*., 2001). The silymarin flavonolignans extracted from Milk thistle seeds protect liver cells against oxidative stress and tissue damage (Cacho *et al*., 1999; Rodriguez-Perez *et al*., 2002; Skottova *et al*., 2004).

While, spring weather in the North-west of Iran practically begs farmers to start planting, it is soil, not air, temperature that governs seed germination. Some sources give minimum temperatures for germination. These are the lowest temperatures at which germination will occur for specific crops. The length of time for germination will be much longer at less-than-optimal temperatures. Planting at lower temperatures also results in greatly reduced germination (Bosland and Votava, 2000).

Soil salinity, if not properly managed, can become a limiting factor for Milk Thistle stand establishment. Excess salinity within the plant root zone has a deleterious effect on plant growth. Plants generally are tolerant during germination but become more sensitive during the emergence and early seedling growth stages (Rhoades *et al*., 1992). It is imperative to keep salinity low in the seedbed during germination and stand establishment. It also is important to realize that salt tolerance data cannot provide accurate, quantitative crop yield losses from salinity for every situation, since actual responses to salinity vary with other conditions, including agronomic factors, soil and water management, climate, crop variety and growth stage (Roundy, 1987). How each of these *Silybum marianum* types responds to soil temperature and salinity conditions is not well understood. Therefore, a replicated study conducted under controlled environmental conditions was performed to observe the interaction of these two critical environmental factors.

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MATERIALS AND METHODS

The Ecology and Medicinal Plants Laboratory conducted the germination tests. Twenty treatments, consisting of 5 salinity levels for each of 4 *Silybum marianum* types with four replications, were arranged in a completely randomized design within each temperature regime (10, 15 and 20°C). Time was considered a split plot with multiple observation times (6, 9, 12, 15, 18 and 21 days after placement). Salinity levels were 1, 3, 5, 7 and 9 dS m^{-1}. There were 4 types of *Silybum marianum* seed used: 2 ecotypes, Pars Abad and Babak, which were collected from North-west of Iran and 2 introduced varieties, Budakalaszi (Hungary) and CN seeds (England). The experimental unit was 100 seeds placed on standard AOSA-approved seed germination blotter paper. Seeds were randomly chosen for each treatment without regard to any physical characteristics or seed viability. The AOSA standard germination test was used as a control or standard for comparison to the imposed incubation temperatures. The standard seed test was run at 20°C in 16 h of darkness followed by 30°C for 8 h of light and used distilled water (electrical conductivity < 0.1 dS m^{-1}) to saturate the blotter and moisten the seeds. Saline solutions were prepared from a mixture of sodium and calcium chloride reagents in a 1:1 equivalent ratio to attain electrical conductivities of 1, 3, 5, 7 and 9 dS m^{-1}. These solutions were used to saturate the germination blotters and moisten the seeds during testing.

The protocol included random selection of 100 seeds per temperature per replicate per each type seeds and saturating the blotter paper with distilled water or saline solution for each combination of salinity, replication and variety within each temperature. Seeds were placed evenly across the test surface and each blotter was then rolled and folded in such a way as to keep the seeds enclosed. The rolled blotter papers were placed in plastic bags labeled with seed type, salinity level, test temperature and replication number. Each bag was then placed in the appropriate temperature-controlled germination chamber.

Six observations were made during the 21 day period to evaluate seed germination. The criteria used to evaluate seed germination were a primary root at least 5 mm long in the root hair zone, elongating hypocotyls above the root hair zone and no lesions or decay from mold.

RESULTS

The results of this study indicated a highly significant ($p = 0.0001$) relationship between salinity and *Silybum marianum* type on germination (Table 1). There were significant interactions between salinity and *Silybum marianum* type and a significant effect of time on germination. There also were significant differences in germination between 10, 15 and 20°C over time. There was no germination during the 21 day test for seed exposed to a constant 10°C temperature treatment, regardless of salinity or seed type. Germination levels at 15°C were

Table 1: Mean percent germination for *Silybum marianum* seed incubated in a growth chamber at either 15 or 20°C and at 5 salinity levels over time

		Germination %									
		15°C					20°C				
		Salinity level (dS m^{-1})					Salinity level (dS m^{-1})				
Silybum marianum type	Day	1	3	5	7	9	1	3	5	7	9
Budakalaszi	6	0.0	0.0	0.0	0.0	0.0	22.5	16.5	13.0	2.5	0.0
	9	0.0	0.0	0.0	0.0	0.0	78.5	69.5	68.0	65.8	34.8
	12	0.0	0.0	0.0	0.0	0.0	85.8	79.8	76.3	80.0	75.5
	15	0.3	0.5	0.5	0.0	0.0	88.3	85.0	79.8	84.8	80.0
	18	8.3	8.5	4.3	4.3	0.0	90.3	85.0	80.5	86.0	81.3
	21	52.3	48.8	38.3	25.3	25.3	89.8	86.5	82.0	85.0	83.5
Pars Abad	6	0.0	0.0	0.0	0.0	0.0	3.3	3.8	1.0	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	58.8	58.3	55.5	54.0	41.8
	12	0.0	0.0	0.0	0.0	0.0	75.3	75.0	71.5	74.8	73.0
	15	2.0	1.8	1.3	2.3	0.3	80.3	78.8	76.3	78.5	77.3
	18	11.0	13.5	17.0	13.0	8.5	80.8	80.0	77.5	80.0	78.5
	21	46.3	42.8	50.0	47.8	50.3	80.0	81.3	79.0	80.0	83.5
Babak	6	0.0	0.0	0.0	0.0	0.0	20.3	12.3	8.0	2.5	0.0
	9	0.0	0.0	0.0	0.0	0.0	84.0	68.5	80.8	73.3	46.3
	12	0.0	0.0	0.0	0.0	0.0	87.3	84.5	83.8	80.5	75.5
	15	2.8	7.8	2.5	1.3	0.0	87.5	87.5	85.5	81.3	77.3
	18	43.0	51.0	42.8	26.8	12.0	87.5	88.0	85.5	81.3	77.3
	21	84.3	84.5	82.5	73.0	67.0	89.3	86.0	83.8	80.5	70.3
CNSeeds	6	0.0	0.0	0.0	0.0	0.0	1.8	2.3	0.8	0.0	0.0
	9	0.0	0.0	0.0	0.0	0.0	80.3	83.8	67.8	51.3	26.3
	12	0.0	0.0	0.0	0.0	0.0	96.8	98.5	95.5	94.3	94.8
	15	0.8	1.5	0.3	1.0	2.0	97.3	99.0	96.3	95.5	95.8
	18	16.3	16.8	14.5	12.0	11.0	97.3	99.0	96.3	95.5	95.8
	21	68.8	67.0	59.3	57.0	42.0	98.0	98.0	95.8	96.8	92.5

Table 2: Mean percentage difference in germination between 20 and 15°C over time for each salinity level

		Salinity level (dS m^{-1})				
Silybum marianum	Day	1	3	5	7	9
Germination Difference (20-15°C, %)						
Budakalaszi	6	22.5	16.5	13.0	2.5	0.0
	9	78.5	69.5	68.0	65.8	34.8
	12	85.8	79.8	76.3	80.0	75.5
	15	88.0	84.5	79.3	84.8	80.0
	18	82.0	76.5	76.3	81.8	81.3
	21	37.5	37.8	43.8	59.8	58.3
ParsAbad	6	3.3	3.8	1.0	0.0	0.0
	9	58.8	58.3	55.5	54.0	41.8
	12	75.3	75.0	71.5	74.8	73.0
	15	78.3	77.0	75.0	76.3	77.0
	18	69.8	66.5	60.5	67.0	70.0
	21	33.8	38.5	29.0	32.3	33.3
Babak	6	20.3	12.3	8.0	2.5	0.0
	9	84.0	68.5	80.8	73.3	46.3
	12	87.3	84.5	83.8	80.5	75.5
	15	84.8	79.8	83.0	80.0	77.3
	18	44.5	37.0	42.8	54.5	65.3
	21	5.0	1.5	1.3	7.5	3.3
CN Seeds	6	1.8	2.3	0.8	0.0	0.0
	9	80.3	83.8	67.8	51.3	26.3
	12	96.8	98.5	95.5	94.3	94.8
	15	96.5	97.5	96.0	94.5	93.8
	18	81.0	82.3	81.8	83.5	84.8
	21	29.3	31.0	36.5	39.8	50.5

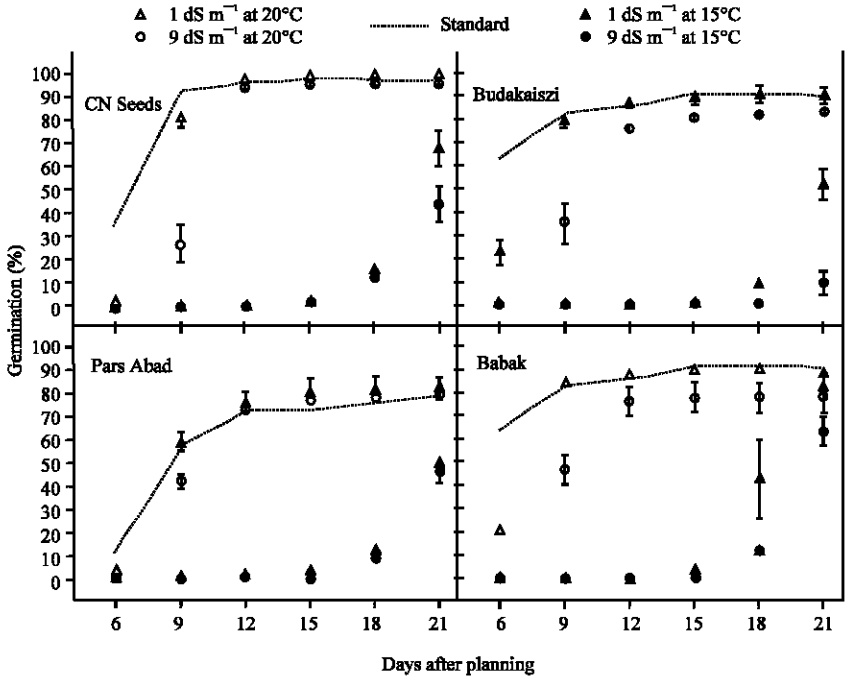

Fig. 1: *Silybum marianum* seed germination for 4 types (Budakalaszi, Pars- Abad, Babak and CN Seeds) as affected by salinity and temperature over time. Standard protocol germination also is plotted as a reference to the temperature treatments,15 and 20°C

drastically lower than those at 20°C during the first 18 days after placement, regardless of salinity or seed type (Fig. 1). After 21 days of incubation, there were some similarities in germination among salinity levels and varieties and some obvious differences (Fig. 1). The results are summarized in Table 2. Babak exhibited very

Table 3: *Silybum marianum* seed germination under standard testing procedures

Day	Budakalaszi	Pars Abad	Babak	CN Seeds
Germination (%)				
6	63.3	12.0	41.5	35.5
9	82.0	57.3	79.3	92.0
12	87.5	71.8	80.3	96.5
15	89.8	74.5	80.8	97.3
18	90.0	76.3	81.3	97.3
21	89.5	80.5	79.5	96.5

little difference in total germination percentage across all salinity levels between 15°C and 20°C <7.5% difference) (Table 3). Budakalaszi had the largest difference in germination among temperatures across all salinity levels (<59.8% difference).

For all tested *Silybum marianum* types subjected to a constant 20°C, 90% of maximum germination occurred within 12 days compared to 0% in 12 days when incubated at 15°C (Fig. 1). Fifteen days elapsed before seeds exposed to a constant 15°C began to germinate. By day 21, seeds subjected to a constant 15°C achieved only 30% of the germination level of seeds subjected to a constant 20°C, at salinity levels at or above 7 dS m^{-1}. Lower salinity levels at 15°C permitted germination to reach a minimum of 58% of the maximum germination achieved at 20°C (Table 3). Only Babak, achieved a comparable 21 day germination percentage at both 15 and 20°C (Fig. 1).

Germination at a constant 20°C was affected by a saline solution of 9 dS m^{-1} during the first nine days of incubation. There was a small depression (<6.3%) in total germination due to the 9 dS m^{-1} solution at 20°C for all types except Pars- Abad. This ecotype had 19% less germination in 9 dS m^{-1} water than in 1 dS m^{-1} water when incubated at 20°C. Budakalaszi, Babak and CN Seeds demonstrated reduced germination with increased salinity levels when incubated at 15°C.

Germination also varied under standard protocols used for evaluating *Silybum marianum* germination. After 21 days of incubation using distilled water, there were similar germination rates between CN Seeds and Budakalaszi (93% average germination) and between Babak and Pars Abad (80% average germination) (Table 3). The 13% difference in average germination between these two groups of *Silybum marianum* was significant. Incubating the seed at 20°C was similar to the standard test procedure (Fig. 1). The largest difference between the constant 20°C incubation and the standard procedure occurred during the first 9 days of incubation (Fig. 1).

DISCUSSION

Temperature had a major impact on seed germination. High levels of salinity affected germination throughout the incubation period. Therefore, it shouldn't be a surprise that growers experience slow germination with early spring plantings when soil temperatures average 15°C. Prolonged contact with a soil environment without germination exposes seeds to diseases, insects, rodents and other detrimental environmental factors. A best management practice would require an average soil temperature of 20°C in the seed zone. Soil temperatures below this level most likely will slow seed germination and predispose seed to biological and environmental damage.

Salinity most likely will have less impact during germination under optimum soil temperature conditions. However, it can affect stand establishment during emergence. The effect of high salinity on seedlings in the cotyledon through true leaf stages is devastating (Rhoades *et al*., 1992).

Under standard the significant difference in germination between *Silybum marianum* vaiieties (Budakalaszi and CN Seeds) and the other 2 ecotypes suggests a difference in *Silybum marianum* seed quality. How the seed is grown and prepared could account for the 13% difference in germination. This study would be improved by using seed lots and *Silybum marianum* types that test similarly under standard germination procedures.

REFERENCES

Bosland, P.W. and E.J. Voltava, 2000. Peppers: Vegetable and *Capsicums* sp. CABI Publishing, London.

Burgess, C.A., 2003. *Silybum marianum* (milk thistle). J. Pharmacy Soc. Wisconsin, pp: 38-40.

Cacho, M., M. Moran, P. Corchete and J. Fernandez-Tarrago, 1999. Influence of medium composition on the accumulation of flavonolignans in cultured cells of *Silybum marianum* (L.) Gaertn. J. Plant Sci., 144: 63-68.

Davork, Z., P. Kosina, D. Walterova, V. Simanek, P. Bachleda and J. Ulrichova, 2003. Primary cultures of human hepatocytes as a tool in cytotoxicity studies: Cell protection against model toxins by flavonolignans obtained from *Silybum marianum*. Toxicol. Lett., 173: 201-212.

Morazzoni, P. and E. Bombardelli, 1995. *Silybum marianum* (*Cardeus marianum*). Fitoterapia, 66: 3-42.

Murphy, J., M. Caban and K. Kemper, 2000. Milk thistle (*Silybum marianum*). http:/www.mcp.edu/herbal/defult.htm.

Rhoades, J.D., A. Kandiah and A.M. Mashali, 1992. The use of saline waters for crop production. Food and Agriculture Organization of the United Nations (FAO), Irrigation and Drainage Paper 48. FAO, Rome, Italy.

Rodriguez-Perez, F., F. Rodriguez-Serrano and D. Toro, 2002. The effects of *Silybum marianum* on the viral load on Hispanic patients with chronic hepatits. AJG., 97: 87-90.

Roundy, B.A., 1987. Seedbed Salinity and the Establishment of Range Plants. In: Frasier, G.W. and R.A. Evans (Eds.). Seed and seedbed ecology of rangeland plants. Washington, DC: Agricultural Research Service, U.S. Dep. Agric., pp: 68-81.

Schuppan D., J.D. Jia, B. Brinkhaus and E.G. Hahn, 1999. Herbal products for liver diseases: A therapeutic challenge for the new millennium. Hepatology, 30: 1099-1104.

Saller R., R. Meier and R. Brignoli, 2001. The use of silymarin in the treatment of liver disease. Drugs, 61: 2035-2063.

Shokrpour, M., M. Moghaddam, S.A. Mohammadi and A. Javanshir, 2007. Genetic properties of Milk thistle ecotypes from Iran for morphological and flavonolignans characters. Pak. J. Biol., Sci., 10 (19): 3266-3271.

Skottova, N., L. Kazdova, O. Oliyarnyk, R. Vecera, L. Sobolova and J. Ulrichova, 2004. Phenolics-rich extracts from *Silybum marianum* and *Prunella vulgaris* reduce a high sucrose diet induced oxidative stress in hereditary hyperglyceridemic rats. Pharmacol. Res., 50: 123-130.