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Araştırma Makalesi

The Effect of L-Tryptophan and Melatonin on Seed Germination of Some Cool Season Vegetable Species Under Salinity Stress

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ABSTRACT

This study was carried out with the objective to evaluate the effects of different concentrations of L-Tryptophan and Melatonin pretreatment on germination behavior of onion, leek, black carrot, and radish seeds exposed to saline stress. Seeds of samples were soaked for 24 hours in distilled water, 50,100, 150 ppm L-tryptophan, and 1.5, 3, and 4.5 µM melatonin solutions at 20° C in darkness. Seeds were placed in Petri dishes and watered to moisten the filter paper with different concentrations of NaCl (0, 150, 300 or 450 mM). The experiment was planned in a completely randomized design with four replications. Maximum germination percentage, germination index, time to complete 50% germination and mean germination time was measured at the end of the study. The results indicated that the effect of pre-treatment was significant on all studied traits in all species except for mean germination time for black carrot seeds. In addition, all germination parameters of seeds were delayed and decreased by salinity increasing from 0 mM to 450 mM NaCl. Overall, all doses of melatonin increased the maximum germination ratio and germination index values slightly under 300 mM NaCl stress conditions. In general, results which obtained from all doses of melatonin were close to each other, whereas increasing doses of L-tryptophan caused a negative effect in some cases.

Keywords: *L-Tryptophan, Melatonin, Salinity, Germination, Tolerance*

L-Triptofan ve Melatonin'in Tuz Stresi Altında Bazı Serin Iklim Sebze Türlerinin Tohum Çimlenmesine Etkileri

ÖZET

Bu çalışma, farklı konsantrasyonlarda L-Triptofan ve Melatonin ön uygulamalarının, tuz stresine maruz bırakılan soğan, pırasa, siyah havuç ve turp tohumlarının çimlenme özellikleri üzerine etkilerini değerlendirmek amacıyla gerçekleştirilmiştir. Örneklere ait tohumlar, 24 saat boyunca, distile su, 50, 100, 150 ppm L-triptofan; 1.5, 3 ve 4.5 µM Melatonin çözeltilerinde karanlıkta ve 20 ° C' de çözeltilerinde bekletilmiştir. Tohumlar petri kaplarına yerleştirilmiş ve filtre kağıtları farklı NaCl konsantrasyonlarında (0, 150, 300 veya 450 mM) su ile

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nemlendirilmiştir. Deneme, dört tekerrürlü tesadüf parselleri şeklinde planlanmıştır. Çalışmanın sonunda maksimum çimlenme yüzdesi, çimlenme indeksi, % 50 çimlenmeyi tamamlama süresi ve ortalama çimlenme süresi değerleri ölçülmüştür. Sonuçlar, ön uygulamaların etkilerinin, siyah havuç tohumlarının ortalama çimlenme süresi hariç, tüm türlerde ve değerlendirilen tüm özellikler üzerinde önemli etkilere neden olduğunu göstermiştir. Buna ek olarak, tuzluluk derecesinin 0 mM' den 450 mM' ye yükselmesi, tohumların tüm çimlenme parametrelerini azaltmış ve geciktirmiştir. Genel olarak, tüm melatonin dozları, 300 mM NaCl stres koşulları altında çimlenme oranını ve çimlenme endeksini bir miktar artırmıştır. Genel olarak, tüm melatonin dozlarından elde edilen sonuçlar birbirine yakın olmasına rağmen, artan L-Triptofan dozları bazı durumlarda olumsuz etkilere neden olmuştur.

Anahtar Kelimeler: *L-Triptofan, Melatonin, Tuzluluk, Çimlenme*

I. INTRODUCTION

Biotic or abiotic agents that adversely affect the growth and development of plants in normal habitat environments are called stress factors [1]. Stress primarily begins with deterioration in metabolic and physiological mechanisms. It causes a decrease in product quality and even death due to damage to plant organs [2]. Salinity stress causes significant losses in yield and quality by changing the soil structure in particular [3]. Besides, salt ions such as Na and Cl can be easily absorbed by plants [4]. Especially, the ionic toxicity caused by the accumulation of high levels of Na^+ causes deterioration in biochemical reactions and inhibits seed germination [5, 6]. Seed germination and seedling growth in salty areas have critical importance for the maintenance of plant life [7].

The high content of melatonin ((N-acetyl-5-methoxytryptamine; MEL) in plants growing under abiotic stress conditions has led to the idea that tolerance to stress factors can be increased through external applications [8]. MEL, a molecule whose presence has been shown in almost all living organisms, first isolated in 1958 from cow brain gland [9]. The amount of MEL in plants not only varies from species to species but also varies between genotypes or varieties within the same species or within different growth stages of the same individuals [10]. Dubbels et al. [11] reported that wild tomatoes *(L. pimpinellifolium*) contained 5 times less MEL compared to commercial tomato varieties (*L. lycopersicum*), and as a result, these varieties were more tolerant to higher ozone levels. Recent studies have documented that MEL is provided significant protection against such environmental stresses as cold, salinity, water, etc. Li et al. [12] stated that the application of external MEL caused an increase in the activity of antioxidant enzymes, especially peroxidase in *Malus hupehensis* grown under salt stress, and it was effective in increasing tolerance. In the study of Korkmaz et al. [13] treatment of pepper seeds with MEL especially in 1 or 5 µM concentrations, significantly improved germination and emergence percentage under chilling stress. Application of MEL has also increased the germination performance of cucumber and *Phacelia tanacetifolia* [14,15].

L-tryptophan (L-T) (3-indolylalanine), has been first discovered in 1901 by the English chemist Frederick Gowland Hopkins, is not only an essential amino acid for plants but also for animals, humans and some bacteria [16]. Although the physiological functions of L-T in plants are still not fully established, some functional effects have been proven. Exogenous application of L-T increases the level of auxin in plant tissues. The positive response of the application of this amino acid to

germination and growth performance of crops has been reported by different researchers in chickpea [17], tomato [18], *Brachiaria ruziziensis* [19], and wheat [20].

Among plant species, varied threshold tolerances and diverse decline ratios of yield or quality are seen and this demonstrates that there is variation in salt tolerance mechanisms [3]. According to Maas [21], the onion and carrot are very sensitive to electrical conductivities values as low as 1.2 and 1.0 dS/m, respectively. In addition, radish has been reported as a salt-sensitive crop by Malcolm and Smith [22]. Leek plants were reported as moderately sensitive to salinity by Kiremit and Arslan [23]. Salinity stress resulted in a yield-response factor (Ky) of 1.481, with a threshold value of 1.2 dS m^{-1} and a decrease in yield slope of 9.62 % per unit increase in soil salinity beyond the threshold value at the end of that study.

To our knowledge, there have not been published any report in terms of improving the seed germination parameters of onion, leek, black carrot, radish including L-T and MEL priming treatments until now. The present work was, therefore, carried out with the objective to survey the effects of different priming treatments with L-T and MEL on seed germination behavior of these cool-season vegetables subject to saline stress.

II. Materials And Method

The study was conducted in the laboratory of the Erciyes University, Agricultural Faculty in 2019. Seed samples of onion (*Allium cepa* L. cv. Pan-88), radish (*Raphanus sativus* cv. Antep), a local variety of black carrot (*Daucus carota* ssp. *sativus* var. *Atrorubens* Alef), and leek (*Allium ampeloprasum* cv. Ala 34) was obtained from a local seller. The seeds were surface sterilized under aseptic conditions with 70 % ethanol for 1 min, followed by 20% commercial bleach (5.25 % sodium hypochlorite) for 10 min. and then rinsed under running water for 2 minutes [24]. Then, seeds were soaked 25 mL of 0 (distilled water), 50, 100, 150 ppm L-T; and 1.5, 3, and 4.5 µM MEL solutions at 20° C in darkness for 24 hours [8]. After treatment, seeds were washed 3 to 4 times with distilled water and were kept to dry on paper towels for 4 hours. Then fifty seeds were placed on double layers of filter paper moistened with 5 mL of distilled water or NaCl solutions in covered 9 cm Petri dishes. In this experiment, 150, 300, and 450 mM NaCl were used as stress conditions. The germination tests were planned according to ISTA [25] rules. A completely randomized design was used with four replications. Although germination experiments in ISTA rules have been limited to fewer days, this duration was considered as 21 days, as it was assumed that salinity stress would extend germination time. The Petri dishes were placed in a germination chamber in the dark at 21˚C. Germinated seeds were recorded daily. 2 mm long radicil formed seeds were considered to be germinated. At the end of the study, the subsequent equations were used to define the effects of L-T and MEL on the germination of seeds [26; 27; 28]:

G-max= Germination rate (%)= (G/T) x 100 (1)
G-index= Germination Index= (1. day G-max / Dt1) + ……..……+ (n. day G-max / Dtn) (2)
G-50 (day) =Time for germination of 50% seeds (3)
MGT= Mean germination time (Day)= [(1.day G x 1) +…..+ (n. day G x n)] / Total G (4)

where T is the total seed number; G number of seeds which were germinated on the day; Gt number of days counted from the beginning of germination. Statistical analysis was conducted using the SPSS

Version 22.0 statistic software package software. The data from the experiment were subjected to a general analysis of variance (ANOVA).

III. RESULTS

The combined analysis of variance indicated that the effect of pre-treatment and salinity was significant for G-max, G-index, G-50, and MGT on all studied species. Furthermore, the interaction between experimental factors was significant on all the traits except for MGT in carrot seeds and in leek seeds for G max (Table 1).

Table 1. *The results of variance analysis for leek, onion, carrot and radish seeds*

		DF	F Ratios			
			G-max	**G-50**	**G-index**	**MGT**
Leek	Pre-treatment	6	4.43 *	169.14 *	6.22 *	11.82*
	Salinity	3	2325.65 *	2640.76 *	1054.83 *	273.31*
	Pre-treatment x Salinity	18	1.76 ^{ns}	138.54 *	4.01 *	17.03*
Onion	Pre-treatment	6	9.38*	101.62*	5.45*	10.97*
	Salinity	3	4174.78*	1097.27*	1045.76*	297.86*
	Pre-treatment x Salinity	18	2.61*	283.81*	2.45*	13.32*
Black Carrot	Pre-treatment	6	4.72*	59.18*	3.78*	0.42^{ns}
	Salinity	3	543.89*	124.70*	368.65*	123.98*
	Pre-treatment x Salinity	18	2.87*	90.19*	2.63*	1.30^{ns}
Radish	Pre-treatment	6	15.53*	6.50*	19.95*	3.52*
	Salinity	3	3972.57*	576.61*	2435.70*	1720.71*
	Pre-treatment x Salinity	18	6.12*	4.43*	8.50*	7.97*
	C Total	83				
	Error	56				

**significant ($p < 0.01$), ns: not significant. F: Freedom, DF: Degree of freedom, G-max: Final germination percentage, G-index: Germination index; G-50: Time for germination of 50% seeds; MGT: Mean Germination Time, C. Total: Corrected Total*

Although the interaction between pre-treatment and salt applications was found to be insignificant for the maximum germination rate, it was found significant for the other characters evaluated in leek seeds. Therefore, the effects of MEL and L-T and salt stress are shown in a separate table (Table 2). In general, the positive effect of MEL on the germination rate was more pronounced than L-T. The highest germination rate was obtained from the application of 3 µM MEL, and the other doses were close, but increased doses of L-T caused a decrease in germination rate. The maximum germination percentage of leek seeds decreased due to increased salt concentration. No germination was observed in 450 mM NaCl salt condition. Generally, germination indexes were reduced by increased salt concentration in leek seeds (Table 3). That effect was more pronounced in MEL or L-T-free treatment than other treatments. The highest germination index values were obtained as a result of 50 ppm L-T, 1.5 µM MEL, and 3 µM MEL treatments. Increased salt concentrations also increased the G-50 values of the seeds. It was found interesting that the application of 1.5 µM and 3 µM MEL in 150 mM and 300 mM NaCl conditions would increase G-50 values. However, this effect was not observed in the salt-free condition. The data obtained from the germination index was similar to the germination rate in leek seeds. The highest germination index value was obtained in 4,5 µM MEL-treated and incubated in non-saline condition seeds. In general, increased salt concentration prolonged the G-50 and MGT duration. Although the differences between MEL and L-T and their doses were generally very small. The treatment of 4.5 µM MEL in the 300 mM salt condition shortened these periods.

Table 2. *The effects of pre-treatments and salinity on maximum germination percentage in leek seeds*

Pre-treatment	**G-Max**	**Salinity**	**G-Max**
Control	40.41 CD	**0 mM NaCl**	94.52 A
50 ppm L-T	47.91 A-C	**150 mM NaCl**	85.48 B
100 ppm L-T	44.58 BC	**300 mM NaCl**	2.62 C
150 ppm L-T	44.16 CD	**450 mM NaCl**	0.00 C
1.5 µM MEL	48.33 AB		
3 µM MEL	48.75 A		
4.5 µM MEL	45.41 A-C		

L-T: L-tryptophan; MEL: Melatonin; G-max: Final germination percentage

Table 3. *Germination features of leek seeds*

Salinity *(mM NaCl)*	**Treatment**	**G-max** *(%)*	**G-Index**	**G-50** *(day)*	**MGT** *(day)*
0	**Control**	85.00	16.40 C	4.00 F	5.74 C-E
	50 ppm L-T	100.00	21.73 A	4.00 F	5.13 E
	100 ppm L-T	93.33	15.03 CD	6.67 D	6.92 A-C
	150 ppm L-T	86.67	21.39 AB	4.00 F	4.48 E
	1.5 µM MEL	100.00	23.16 A	4.00 F	4.73 E
	3 µM MEL	100.00	23.29 A	3.33 G	4.72 E
	4.5 µM MEL	96.67	19.41 B	3.33 G	5.45 DE
150	**Control**	76.67	11.05 F	7.00 D	7.00 A-C
	50 ppm L-T	91.67	12.91 D-F	7.00 D	7.27 AB
	100 ppm L-T	85.00	12.13 E-F	7.00 D	7.20 AB
	150 ppm L-T	86.67	13.42 DE	8.00 C	6.67 A-D
	1.5 µM MEL	88.33	12.02 E-F	8.33 C	7.44 AB
	3 µM MEL	85.00	12.20 EF	8.00 C	7.07 A-C
	4.5 µM MEL	85.00	13.47 DE	6.00 E	6.61 B-D
300	**Control**	0.00	-	-	-
	50 ppm L-T	0.00	-	-	-
	100 ppm L-T	1.00	-	-	-
	150 ppm L-T	3.33	0.47 G	12.00 A	4.67 E
	1.5 µM MEL	5.00	0.66 G	10.00 B	7.67 AB
	3 µM MEL	10.00	1.27 G	12.00 A	8.00 A
	4.5 µM MEL	1.00	-	-	-
450	**Control**	0.00	-	-	-
	50 ppm L-T	0.00	-	-	-
	100 ppm L-T	0.00	-	-	-
	150 ppm L-T	0.00	-	-	-
	1.5 µM MEL	0.00	-	-	-
	3 µM MEL	0.00	-	-	-
	4.5 µM MEL	0.00	-	-	-

L-T: L-tryptophan; MEL: Melatonin; G-max: Final germination percentage, G-index: Germination index; G-50: Time for germination of 50% seeds, MGT: Mean Germination Time

Table 4. *Germination features of onion seeds*

Salinity *(mM NaCl)*	**Treatment**	**G-max** *(%)*	**G-Index**	**G-50** *(day)*	**MGT** *(day)*
0	**Control**	95.00 A-C	16.83 C-E	5.33 HI	7.02 D-G
	50 ppm L-T	100.00 A	16.39 C-F	5.00 HI	6.72 E-G
	100 ppm L-T	100.00 A	19.57 A-C	4.33 I	6.68 E-G
	150 ppm L-T	100.00 A	20.39 A-C	5.00 HI	6.65 E-G
	1.5 µM MEL	100.00 A	17.69 B-D	5.00 HI	7.20 D-G

	3 µM MEL	98.33 A	21.03 AB	6.33 GH	6.36 FG
	4.5 µM MEL	100.00 A	22.22 A	5.67 G-I	5.77 G

Table 4. *(Continued)*

150	**Control**	85.00 E	10.71 G	7.00 FG	8.27 C-E
	50 ppm L-T	91.67 B-D	12.05 G	6.00 GH	7.98 D-F
	100 ppm L-T	90.00 C-E	10.86 G	9.00 DE	8.66 CD
	150 ppm L-T	88.33 DE	11.74 G	6.00 GH	7.72 D-F
	1.5 µM MEL	100.00 A	12.81 E-G	7.00 FG	8.23 C-E
	3 µM MEL	96.67 AB	14.44 D-G	8.33 EF	7.67 D-F
	4.5 µM MEL	100.00 A	12.64 FG	6.33 GH	8.25 C-E
300	**Control**	0.00 H	-	-	-
	50 ppm L-T	13.33 F	1.19 H	12.00 AB	11.22 AB
	100 ppm L-T	5.00 GH	0.42 H	12.00 AB	12.00 A
	150 ppm L-T	6.67 G	0.58 H	11.00 BC	11.33 AB
	1.5 µM MEL	13.33 F	2.11 H	11.33 A-C	10.77 AB
	3 µM MEL	15.00 F	1.52 H	12.67 A	10.77 AB
	4.5 µM MEL	18.33 F	1.91 H	10.33 CD	9.89 BC
450	**Control**	0.00	-	-	-
	50 ppm L-T	0.00	-	-	-
	100 ppm L-T	0.00	-	-	-
	150 ppm L-T	0.00	-	-	-
	1.5 µM MEL	0.00	-	-	-
	3 µM MEL	0.00	-	-	-
	4.5 µM MEL	0.00	-	-	-

L-T: L-tryptophan; MEL: Melatonin; G-max: Final germination percentage, G-index: Germination index; G-50: Time for germination of 50% seeds, MGT: Mean Germination Time

Generally, germination percentages were reduced by increased salt concentration in onion seeds (Table 4). In 150 mM NaCl condition, maximum seed germination was obtained in the 5 µM MEL treatment (100 %). When seeds were incubated in 300 mM NaCl condition, germination percentage was more adversely affected than 150 mM NaCl. No germination was observed in 450 mM NaCl salt treatment. Although both MEL and L-T significantly affected germination under stress conditions, the positive effect of MEL was stronger than L-T. The mean time to germination was also significantly affected by salinity concentration.

The effects of pre-treatments on black carrot seeds are shown in Table 5. The highest germination rate was obtained in salt-free condition and in non-treated seeds (98.33 %). The increased dose of MEL reduced the germination rate as in L-T. In contrast to onion, radish and leek seeds, germination seen with MEL and L-T application in 450 mM salinity conditions in black carrot seeds. In this salinity condition, no germination was observed in the control group and 50 ppm L-T treatment. The highest germination index value was observed in maximum doses of both MEL and L-T under non-saline conditions. In the most severe stress condition, all MEL doses gave the highest germination index values. Days to reach 50% of final germination percentage (G-50) of carrot seeds ranged from 2.00 days (0 salinity/50 ppm L-T) to 9.67 days (450 mM salinity/100; 150 ppm L-T, and all MEL treatments). Generally, salinity increased MGT, especially in untreated seeds.

Table 5. *Germination features of black carrot seeds*

Salinity *(mM NaCl)*	**Treatment**	**G-max** *(%)*	**G-Index**	**G-50** *(day)*	**MGT** *(day)*
0	**Control**	98.33 A	29.75 AB	3.33 H-J	3.76
	50 ppm L-T	95.00 AB	32.42 A	2.00 K	3.28

	100 ppm L-T	93.33 AB	29.00 AB	3.00 IJ	3.54
	150 ppm L-T	90.00 AB	31.25 A	2.67 JK	3.24
	1.5 µM MEL	96.67 AB	26.08 BC	3.00 IJ	3.87
	3 µM MEL	93.33 AB	30.00 AB	3.00 IJ	3.43
	4.5 µM MEL	93.33 AB	33.50 A	3.00 IJ	3.17

Table 5. *(Continued)*

150	**Control**	61.67 DE	14.17 EF	4.33 E-G	4.41
	50 ppm L-T	93.33 AB	20.46 D	4.00 F-H	5.16
	100 ppm L-T	95.00 AB	21.59 CD	5.00 DE	5.09
	150 ppm L-T	73.33 CD	19.71 D	3.67 G-I	4.42
	1.5 µM MEL	88.33 AB	22.92 BC	3.33 H-J	4.50
	3 µM MEL	85.00 BC	19.67 D	3.00 IJ	5.02
	4.5 µM MEL	90.00 AB	19.71 D	4.67 D-F	5.06
300	**Control**	43.33 FG	7.63 G	8.33 B	6.59
	50 ppm L-T	66.67 DE	19.09 D	7.00 C	4.32
	100 ppm L-T	61.6E DE	12.76 F	4.67 D-F	5.91
	150 ppm L-T	56.67 E	11.50 FG	5.33 D	6.18
	1.5 µM MEL	55.00 ED	9.71 FG	4.33 E-G	4.47
	3 µM MEL	66.67 DE	18.25 DE	5.00 DE	4.85
	4.5 µM MEL	41.67 G	9.50 FG	5.00 DE	5.29
450	**Control**	-	-	-	-
	50 ppm L-T	-	-	-	-
	100 ppm L-T	3.33 H	0.67 H	9.67 A	0.27
	150 ppm L-T	3.33 H	0.67 H	9.67 A	0.28
	1.5 µM MEL	10.00 GH	1.42 GH	9.67 A	1.25
	3 µM MEL	8.33 GH	1.42 GH	9.67 A	0.76
	4.5 µM MEL	8.33 GH	2.16 GH	9.67 A	0.68

L-T: L-tryptophan; MEL: Melatonin; G-max: Final germination percentage, G-index: Germination index; G-50: Time for germination of 50% seeds, MGT: Mean Germination Time

Table 6 presents the effect of L-T and MEL on seed germination of radish at various salinity conditions. In the most severe salt stress condition (450 mM), no seed was germinated as in leek and onion. In the 150 mM salt condition, which is the lowest level of stress, all pre-treatments have a positive effect on germination. In the 300 mM salt stress condition, the effect of MEL on maximum germination rate was more pronounced than in L-T. Under this condition, the maximum germination rate of after 100 ppm L-T was reduced slightly. The highest value of germination index (64.17) was registered for the treatment of 4.5 MEL /non-saline condition, and, then values decreased as salinity stress conditions became more severe.

Table 6. *Germination features of radish seeds*

Salinity ***(mM NaCl)***	**Treatment**	**G-max** *(%)*	**G-Index**	**G-50** *(day)*	**MGT** *(day)*
0	**Control**	95.0 AB	48.57 G	2.00 D	2.42 EF
	50 ppm L-T	100.00 A	58.75 C-E	2.00 D	2.10 GJ
	100 ppm L-T	100.00 A	56.94 C-E	2.00 D	2.13 G-I
	150 ppm L-T	96.67 A	59.99 B-D	2.33 CD	1.94 I-K
	1.5 µM MEL	100.00 A	55.28 EF	2.00 D	2.23 F-H
	3 µM MEL	100.00 A	62.91 AB	2.00 D	1.83 JK
	4.5 µM MEL	100.00 A	64.17 A	2.00 D	1.75 K
150	**Control**	90.00 BC	41.25 H	2.67 BC	2.64 DE
	50 ppm L-T	100.00 A	56.11 D-F	2.00 D	2.13 G-I
	100 ppm L-T	96.67 A	52.09 FG	2.33 CD	2.36 FG
	150 ppm L-T	100.00 A	56.53 C-E	2.00 D	2.10 G-J
	1.5 µM MEL	100.00 A	60.28 A-C	2.00 D	1.97 H-K
	3 µM MEL	100.00 A	60.28 A-C	2.00 D	1.92 I-K

	4.5 µM MEL	100.00 A	50.56 G	2.00 D	2.32 FG

Table 6. *(Continued)*

300	**Control**	70.00 E	27.78 J	3.67 A	2.87 D
	50 ppm L-T	88.33 CD	25.19 J	4.00 A	3.90 A
	100 ppm L-T	96.67 A	34.66 I	3.67 A	3.94 A
	150 ppm L-T	83.33 D	26.53 J	4.00 A	3.67 BC
	1.5 µM MEL	100.00 A	34.47 I	2.33 CD	3.53 C
	3 µM MEL	100.00 A	34.55 I	3.00 B	3.60 C
	4.5 µM MEL	95.00 AB	35.67 I	3.00 B	3.58 C
450	**Control**	-	-	-	-
	50 ppm L-T	-	-	-	-
	100 ppm L-T	-	-	-	-
	150 ppm L-T	-	-	-	-
	1.5 µM MEL	-	-	-	-
	3 µM MEL	-	-	-	-
	4.5 µM MEL	-	-	-	-

L-T: L-tryptophan; MEL: Melatonin; G-max: Final germination percentage, G-index: Germination index; G-50: Time for germination of 50% seeds, MGT: Mean Germination Time

IV. DISCUSSION

The result of our germination experiments showed high leek seed germination capacity at the non-saline condition. Specht and Keller [29] indicated that germination percentage of various *Allium* subgenus seeds were observed as 87% in 0 mM, 75 mM, and 150 mM NaCl and the lowest percentage (68.4 %) in 225 mM salinity condition. Likewise, Guenaoui et al. [30] stated that that germination was inhibited by an increase in both temperature and salinity in leek seeds. Also, delay of germination and the time to half of the germination significantly increased in response to salt and temperature. Kiremit and Arslan [23] reported that leek plant stems fresh weight, stem length, stem diameter, stem dry weight, leaf dry weight, leaf fresh weight, leaf length, root fresh weight, and root dry weight decreased with increases in the salt concentration of irrigation water. These results are in agreement with the finding of this study.

In this study, different levels of salinity have a significant effect on all germination parameters of onion seeds. Strong decreases in maximum germination ratios and germination indexes were observed mainly at higher levels of salt concentration compared to control. According to the analysis of variance, interactions of salinity and pre-treatments are significant for all the measured parameters. Gunisetty and Khateef [31] reported that high NaCl concentrations have a negative effect on the germination parameters of onion seeds as well as the physiological quality of onion seedlings. In their study, the germination rates of four different onion varieties were in the 81.67-93.33% range in salt-free media, whereas this ratio decreased to 11.66-43.67 % in 200 mM salt-containing media. These results are similar to our findings.

The increase of the saline concentration in the solution produced a significant reduction in black carrot seed germination capacity. The results obtained for black carrot seeds in this study agree with the report of Bolton and Simon [32]. In their study, carrot plant introductions from the U.S. Department of Agriculture (USDA) National Plant Germplasm System representing 41 different countries, inbred

lines from the USDA Agricultural Research Service, and widely grown commercial hybrids were screened for tolerance under salinity stress and nonstress conditions (150 and 0 mM NaCl, respectively) by measuring the various germination parameters. All salt tolerance measurements differed significantly between accessions; absolute decrease in the percent of germination ranged from −4.2 % to 93.0 %; inhibition index ranged from −8.0 % to 100.0 %; relative salt tolerance ranged from 0.0 to 1.08. The interesting point in this study is that; PI 256066, PI 652253, PI 652402, and PI 652405 code number accessions collected from Turkey were found to be tolerant of salt. These results confirm the findings obtained from our study. In our study, a local variety of black carrot was used as plant material, and only these seeds could germinate under 450 mM salinity conditions with MEL and L-T application.

This study showed that increasing NaCl concentration decreased the germination percentage in radish seeds. The results of this study clearly showed that priming with all doses of MEL (1, 3, 4.5 μM) and 100 ppm L-T improved germination percentage and rate, according to the control in spite of the use of high concentrations of NaCl (300 mM). Kaymak et al. [33] indicated that applications of bio-priming with bacteria strains significantly improved the percentage of radish seed germination under saline conditions.

In this study, MEL significantly reversed the inhibitory effects of NaCl on germination of leek, onion, radish, and black carrot seeds. This effect was more pronounced in radish seeds and 300 mm salt conditions. At this salt concentration, 100% of the 1 μM MEL applied seeds were germinated. However, this ratio was 70% in untreated seeds. MEL has also shown a positive effect in carrot seeds under salt stress. Treatment of 3 μM MEL increased the germination rate from 43.33 % to 66.67 % in carrot seeds under 300 mM NaCl conditions compared to the control group. In onion and leek seeds, no germination has not been observed in untreated seeds under 300 mM NaCl level. However, 18.33% germination was observed in 4.5 μM MEL treated onion seeds. Similarly, 10 % of germination was measured in 3 μM MEL applied leek seeds.

A lot of studies have shown the important and indispensable roles that MEL plays in increasing salt tolerance in various plant species. However since no reports have been published so far to investigate the effects of MEL and L-T on leek, onion, carrot and radish seeds, the comparison is possible only with other species. Zhang et al. [14] indicated that the percentage of germination of cucumber seeds increased with MEL pretreatment in salt stress conditions. The MEL content significantly increased during the first 14 hr of seed germination under normal conditions and then decreased to a relatively steady level while NaCl stress significantly inhibited MEL biosynthesis during seed germination. Seeds primed in 1 μM MEL for 24 hr had MEL levels approximately nine-fold higher than unprimed seeds. In these seeds, during the germination, MEL content decreased while alleviating the inhibitory effects of high salinity. Although the effect of MEL on germination rate was 10 % in leek seeds at 300 mM salt conditions, the effect of L-T was very low. Manchester et al. [34] put forward that the high amount of MEL defined in seeds likely provides anti oxidative defense in a dormant and more or less dry system, in which enzymes are poorly effective and cannot be upregulated. Thus in seeds, MEL may be necessary for protecting germ and reproductive tissues of plants from extreme environmental conditions.

Under salt stress conditions, seed germination and root development might be limited, plant growth might be depressed, and net photosynthetic rate and chlorophyll amount might be decreased, whereas pre-treatment with exogenous MEL may be allowed plants to maintain robust roots, reduce growth inhibition [35].Different concentrations (50, 100, 150 and 200 μmol/L) of MEL increased the biomass,

chlorophyll content and antioxidant enzyme activity of radish seedlings under salt stress in the study of Jiang et al. [36]. At the end of the study, it was reported that when the concentration of MEL was 100 μmol/L, the biomass, chlorophyll content and antioxidant enzyme activity of radish seedlings got the maximums.Cucumber seeds treated with MEL display an improved germination rate during chilling stress in the study of Posmyk et al. [37]. Also, pre-treatment with MEL attenuated apoptosis induced by cold temperature in cultured carrot suspension cells [38].

There are few studies investigating the effects of L-T on plants. Hussein et al. [39] conducted a study to evaluate the effects of spraying with L-T and nicotinic acids on onion plants grown under varying degree of salinity stress. It was reported that endogenous application of L-T and nicotinic acids significantly increased the top height and fresh weight, and bulb dry weight. These useful effects were greater in the plants sprayed with nicotinic acids as compared to those of the plants received L-T spray. At the lower salinity level (3000 ppm), L-T was more effective than nicotinic acids in mitigating the negative effects of salinity stress.

IV. Conclusion

Although several studies about the effect of MEL and L-T on germination of different plant species were conducted under stress conditions, this is the first study in which these are tested together. Also, these cool-season vegetable species were subjected for the first time in such a study.

Evidence from the study indicates that MEL treatment significantly increased the percentage of germination and germination indexes at 300 mM salinity conditions in all species. Also, treating seeds with L-T solutions increased these parameters at the same condition, but this effect less than MEL. In general, the lowest dose of L-T was sufficient for a positive effect, otherwise increased doses would be caused a negative effect. The species used in the study are generally sensitive to salinity. Germination rates were expected to be lower, considering the tried doses. However, in all four species, untreated seeds were able to germinate at certain rates at 150 mM salinity. This can be interpreted as a positive feature of the "cultivars" choosen. As already mentioned in the "Materials and Methods" section, all cultivars were developed in Turkey, and have a wide adaptability.

These ideas need to be examined further by checking environmental factors, such as temperature, light, humidity and so on. In addition, the doses of MEL and L-T which subjected to this study have been mainly selected by other species. Therefore, it may be necessary to examine the doses in more detail in progressive studies.

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