THE EFFECTS OF APPLYING NATURAL PLANT ANTIFREEZE UNDER LOW TEMPERATURE CONDITIONS ON LETTUCE (*LACTUCA SATIVA* L.) YIELD AND QUALITY

PIRINC, V.[1*] – ALAS, E.[2]

[1]*Agriculture Faculty, Horticulture Department, Dicle University, Diyarbakir, Turkey*

[2]*Gap International Agricultural Research and Training Center, Diyarbakir, Turkey*
(e-mail: edip.alas@tarimorman.gov.tr)

**Corresponding author*
e-mail: vedpir@dicle.edu.tr; phone: +90-412-241-1000/8576; fax: +90-412-241-1048

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Abstract. Arctic fishes contain a type of “antifreeze compound” a protein that protects them from cold damage. “Natural plant antifreezes” have been recently developed to offer the same protection to plants. The aim of this study was to investigate the effects of applying this new antifreeze to lettuce grown under different growing systems. Lettuce (*Lactuca sativa* L.) was grown under four different growing systems; open-field, mulch, low-tunnel, low-tunnel + mulch systems and open-field control (antifreeze not applied). The plant properties, such as height, head circumference, number of leaves, root length, root collar diameter, mean plant weight, and yield were examined. The measurements and statistical analyses revealed significant differences. Our results suggested that applying antifreeze to the plants during autumn under different growing conditions improved lettuce yield and number of leaves per plant in addition to other plant properties. Heavy metal and nitrogen/protein analyses after harvest revealed that the elements contained in the plant antifreeze increased the amounts of nitrogen/protein and other beneficial minerals such as magnesium, iron, and zinc in the plants. These results also suggested that natural plant antifreeze applied under greenhouse growing systems can be beneficial to lettuce cultivation under low temperature conditions. Thus, it is possible to grow lettuce, which is known to have a short vegetation period, in autumn under low temperature conditions in most of the regions of Turkey and or other countries.

Keywords: *Lactuca sativa, temperature stress, low tunnel, growing systems, mulch*

Introduction

As salad ingredients, cucumber and lettuce are among the most commonly consumed vegetables throughout the world. They are annual cold-climate plants that are available on the market year-round; however, they have a short growing period of 2–3 months. These plants can be produced successively during an entire year using rehabilitated varieties that are adapted to all seasons under open-field and greenhouse growing conditions (Aybak, 2002; Güngör and Çukadar, 1999).

Lettuce (*Lactuca sativa* L.) is fondly consumed worldwide and is an irreplaceable ingredient in salads. It is an annual cold-climate vegetable with an optimum temperature range of 15–18 °C. Lettuce flowers quickly in summer because of the warm weather and is resistant to temperatures below 0 °C for only a short period of time. During this period, there are between 6 and 10 leaves on each plant. Winter varieties can resist temperatures between 0 and -5 °C for 5–10 d and can resist temperatures at -10 °C for 1–3 d (Günay, 1981). Thus, the most important limiting factor for growing lettuce is low temperatures during autumn and winter and high temperatures during summer. Different measures have been taken to eliminate this factor in different ecologies, such

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as that of Diyarbakir, Turkey, but natural and effective growing methods should be preferred.

The occurrence of low temperatures is one of the most important environmental constraints limiting plant productivity. In order for plants to survive at low temperatures, it is their tolerance to block the formation of ice in plant tissue. Extracellular ice, in addition to mechanical action, can subject plant cells to significant freezing dehydration. Plants have developed different strategies to cope with these low temperatures, such as freeze avoidance and freeze tolerance (Buchner et al., 2020).

Cold-tolerant plants survive in subzero temperatures from the growth of extracellular ice in their tissues. Although the mechanism of cold tolerance in plants is complex, one component involves the secretion of antifreeze proteins (AFPs) into the apoplast of leaves and crowns (Griffith et all., 2005). Antifreeze proteins are called various proteins that bind to ice crystals and stop their growth (Gupta and Deswal, 2014). Researchers have demonstrated the roles of these proteins, which has led to their collective identification as “antifreeze proteins” (Vincents et al., 2008).

The most important factor limiting the cultivation of lettuce, which is an important vegetable for Diyarbakır, in the autumn period, is the early autumn and winter frosts. The current study investigated how to improve the adaptability of lettuce, to autumn and winter growing conditions in Diyarbakir using specific methods.

The effects of applying antifreeze to lettuce in open-field, low-tunnel, mulch, and low-tunnel + mulch growing systems and open-field control (antifreeze not applied) were examined to determine the plant’s tolerance to low temperatures during autumn and winter.

Materials and methods

The study was conducted in 2015 during the vegetation period in the Research and Application area of the Horticultural Department Agriculture Faculty of Dicle University Diyarbakir, Turkey (*Fig. 1*). Seedlings of the lettuce variety of Bitez, were used as the plant material. The seedlings were planted on September 14, 2015, on plots that were prepared as open-field, low-tunnel, mulch, and low-tunnel + mulch systems and open-field control (antifreeze not applied) 30 cm between rows and 25 cm within rows. A transparent polyethylene cover was used both as the mulch material and low-tunnel cover. The study was conducted according to randomized split blocks design with three repetitions. The experiment were established with 8 parcels and each parcel included 30 plants and total 720 seedlings were used.

Cropaid NPA, a commercial biological antifreeze used to protect plants from cold and frost, was applied to the plants in each growing system; the control group comprised plants grown in an open field without antifreeze. Cropaid NPA contains $10^7/\text{cm}^3$ *Thiobacillus* spp. bacteria (*T. ferrooxidans*, *T. thiooxindans*, and *T. thioparus*) and 50 different types of mineral matter, pyruvic acid, oxaloacetic acid, and rusticyanin. The bacteria protect the plants from the cold and provide them with certain advantages by producing biological compounds, such as antifreeze amino acids, antifreeze proteins, vitamins, enzymes, and natural organometallic compounds (chelates).

The dosage of the antifreeze application was adjusted to 400–450 g Cropaid NPA per 100 L water and stirred, after which the antifreeze was micronized and sprayed onto the plant until thoroughly wet. The antifreeze was first applied on October 19 and again on November 24 (*Fig. 2*).

Figure 1. *Photo of the experiment area*

Figure 2. *Photo applying plant antifreeze*

The soil samples collected from the experiment area in accordance with the conditions were analyzed. The analyses showed that the soil was appropriately fertilized to meet the nutritional needs of the plants. The water requirements of the plants were met using drip irrigation pipes together with considering seasonal rainfall. The climate data for the vegetation period from September through December in Diyarbakir were obtained from the General Directorate of Meteorology as shown in *Table 1* and *Figure 3* (Anonymous, 2016a).

Table 1. *Temperature values of Diyarbakır in 2015 year*

Months	Average the highest temperature (°C)	Average the lowest temperature (°C)	Average temperature (°C)
September	36.8	17.3	26.9
October	27.7	11.8	18.1
November	18.4	2.9	9.5
December	12.4	-2.1	3.9

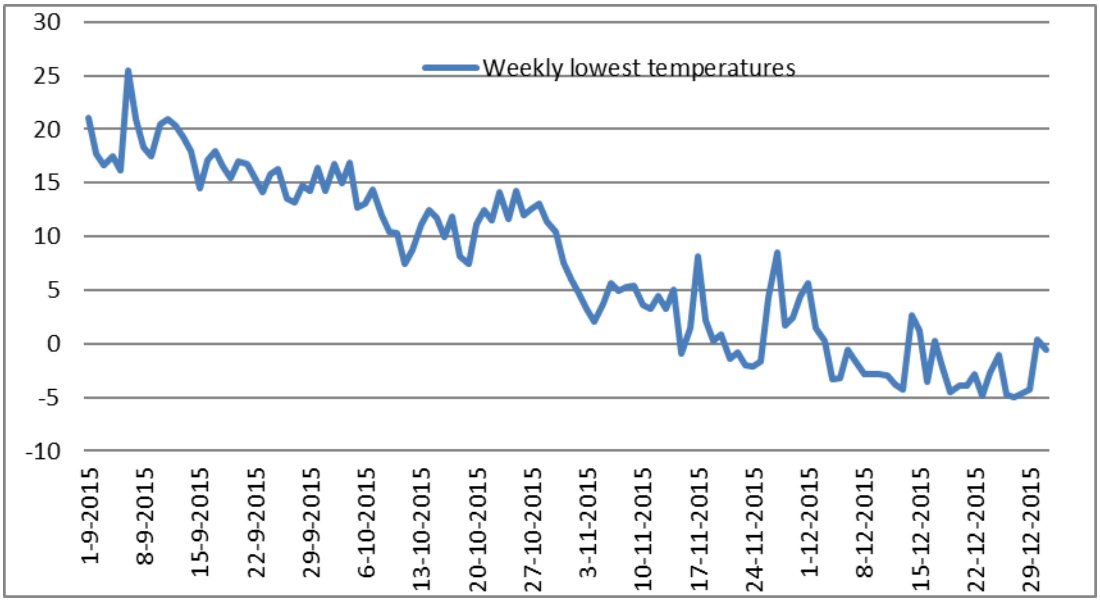

Figure 3. *Diyarbakır the lowest temperatures of lettuce vegetation period in 2015*

Investigated properties

Measurements of the specific plant properties were taken on each of 15 plants collected from each plot on harvest day (December 6), the period at which the plants reached optimum growth. The properties and method of measurement were as follows:

Plant height (cm) measured from the soil level to the uppermost point of the plant using a measuring tape.

Fresh plant weight (g) was measured using an electronic scale after removing the roots.

Plant head circumference (cm) was measured using a measuring tape.

Root collar diameter (mm) was measured using digital calipers.

Root length (cm) was measured by opening pit the crown projections of the plants using a shovel and pulling the plants up from the soil with their roots. It was removed with as little damage as possible to the roots of the plant.

The number of leaves were pulled off each harvested plant and counted to get a total per plant.

Total plant yield (kg/m^2) was measured by weighing the harvested plants using an electronic scale and determining the number of plants per 1 m^2.

After harvesting. the plants were sun dried and kept in an oven at 70 °C for 6 h to remove the moisture. The plants were then powdered using a blender and kept in sunlight under laboratory conditions. The powdered lettuce samples were numbered and prepared for analyses of mineral matter. protein. and nitrogen.

The powdered lettuce samples were then treated with hydrogen peroxide and nitric acid. diluted with water. and dissolved in the Microwave Mars Xpress device. The elemental analyses of the dissolved lettuce samples were conducted using an inductively coupled plasma–optical emission spectrometer.

To investigate the effects of the antifreeze on the nutrient content of the lettuce samples, the protein and nitrogen in the dried lettuce leaves were analyzed using the Turbo N 4040 instrument (Costech Analytical Technologies).

The data obtained was carried out using the Oneway Anova Tukey test in the SPSS 23 statistical package program.

Results and discussion

The results of plant height, plant diameter, head circumference, root collar diameter, and root lengths of the plants grown under different growing conditions were given in *Table 2*. The effects of the antifreeze application on plant height, plant diameter, head circumference, root collar diameter, and root length were significant at a level of 1% in all growing systems ($P \leq 0.001$).

The maximum heights were obtained in the open-field (with antifreeze) plot (26.15 cm) and control plot (26.37 cm), while the minimum heights were obtained in the mulch plots.

Table 2. *Effect of antifreeze application on plant growth properties*

Growing systems	Plant height (cm)	Plant diameter (cm)	Root lengths (cm)	Head circumference (cm)	Root collar diameter (mm)
Low-tunnel	24.8ab	28.6a	12.3a	11.8a	23.2ab
Mulch	23.4b	26.0c	9.4b	2.1c	22.9ab
Open-field	26.1a	27.8ab	8.9b	4.5b	24.3a
Low-tunnel + Mulch	24.5ab	26.6bc	7.5c	10.9a	21.3b
Open field (control)	26.3a	28.8a	8.9b	5.8b	24.5a
	$P \leq 0.001$ **	$P \leq 0.001$ **	$P \leq 0.001$ **	$P \leq 0.001$ **	$P \leq 0.01$ *

Plant diameter varied between 26.0 and 28.8 cm. The largest diameters were found in the open-field and low-tunnel systems, while smallest was found in the mulch system. These diameters were similar to those found in a 2014 study (Güvenç, 2004) (i.e. 29.70–31.73 cm).

The statistical analyses of the effects of antifreeze on head circumference showed that the low-tunnel and low-tunnel + mulch systems had the largest head circumferences (11.8 cm), while the mulch system had the smallest.

The largest root collar diameters were found in the open-field and control plots. while the smallest were found from the low-tunnel + mulch system. The longest roots were found in the low-tunnel system. while the shortest were found in the low-tunnel + mulch system.

Table 3 lists the effects of the antifreeze on lettuce growth and yield. The effect of antifreeze on the mean weight of the lettuce was significant at a level of 5% ($P \leq 0.01$). The heaviest plant. with a weight of 773.3 g. was found in the low-tunnel plot; the lightest plant was found in the mulch system. These results are similar to those found in other studies (Güvenç et al., 2004; Koçar, 2001; Ünlü et al., 2006).

The effect of antifreeze on the number of leaves and yield was not statistically significant. The most leaves per plant (49.4) was found in the low-tunnel system. while the least number (36.4) was found in the control system. The total leaf number determined (58.3) is similar to those found in our study (Tüzel et al., 2011). Although the results are not statistically significant. the use of antifreeze did not increase the number of leaves per plant and resulted in a partial increase in yield (*Table 3*).

Table 3. *Effects of the antifreeze on lettuce growth and yield*

Growing systems	**Mean fresh weight of the lettuce (g)**	**Number of leaves (leaves per plant)**	**Yield (kg/m^2)**
Low-tunnel	773.3a	49.4	9.3
Mulch	596.6b	39.2	7.2
Open-field	736.6ab	47.4	8.8
Low-tunnel + Mulch	653.3ab	48.2	7.8
Open-field (control)	710.0ab	36.4	8.5
	$P \leq 0.01$*	*n.s	*n.s

*not significant

Table 4 shows the results of the laboratory analyses for the effects of antifreeze on the nutrient content in the lettuce leaves. The results of the analysis of the amount of lead in the lettuce plants grown under different antifreeze-applied growing systems were statistically significant ($P \leq 0.01$). In the antifreeze-applied low-tunnel. mulch. open and low-tunnel + mulch growing systems. the amount of lead decreased compared to that in the control plants that were grown in an open field without antifreeze (*Table 5*). Another study on the elemental content in Yedikule lettuces. have found that the magnesium (Mg), sodium (Na), zinc (Zn), and copper (Cu) contents are similar to those found in our study (Demir et al., 2003). The effects of antifreeze on arsenic. calcium. cadmium. Cu, iron, Mg, Na, selenium, and Zn contents were not statistically significant.

Table 4. *Effect of antifreeze application on nutrient content (mg kg-1)*

Elements quantity average	**Low-tunnel**	**Mulch**	**Open-field**	**Low-tunnel + mulch**	**Open-field (control)**
As	0.04	0.42	0.44	0.28	0.31
Ca	3628	2870	3353	3039	3454
Cd	0.13	0.18	0.14	0.22	0.11
Cu	10.60	15.54	12.31	15.00	17.83
Fe	411.1	556.2	646.1	308.8	439.8
Mg	2105	1640	2049	1693	1862
Na	1315	1063	1132	1139	1679
Pb	0.00a*	0.54b*	0.28ab*	0.47ab*	0.61b*
Se	0.36	0.04	0.07	0.00	0.07
Zn	16.12	17.07	19.80	17.63	18.69

Moreover. the analyses showed that the elements in the antifreeze increased the amounts of nitrogen. protein. and beneficial minerals. such as Mg, Fe, and Zn in the plants. *Table 5* provides the results of the analysis of nitrogen and protein in the plants.

Antifreeze application increased the amount of nitrogen in the plants grown in the open-field system compared to those grown in the control system without antifreeze (*Table 5*). This can be attributable to increased nitrogen binding resulting from increased activity of the *Rhizobium* caused by the microorganisms in the antifreeze.

Table 5. *Result of lettuce plants nitrogen protein analysis*

Growing systems	Nitrogen (mg)	Protein (mg)
Low-tunnel	156.8	980
Mulch	155.2	970
Open-field	196.8	1230
Low-tunnel + Mulch	238.4	1490
Open-field (control)	153.6	960

The multiplication of the nitrogen amount with 6.25 gives the protein amount (Anonymous, 2016b)

Conclusion

The consumption of lettuce in Diyarbakir, Turkey is high and the region must meet its demand for lettuce from outside sources. The most limiting factors in lettuce growing within the region are bolting from increased temperatures in spring and cold damage resulting from decreased temperatures in autumn. The use of varying growing systems. such as low tunnel. mulch. and low-tunnel + mulch. is recommended for growing lettuce in autumn and under low-temperature conditions. Çimen et al. (2010) in their study. Diyarbakir ecology emphasized that the growth of plants stops due to low temperature in autumn period and unmarketable products occur. However, there were no significant among applications but according to lettuce quality and marketable yield in low tunnel parcel were higher than others in our study. Different researchers (Koçar, 2001; Ünlü et al., 2006; Demir et al., 2003) have reported that the use of different growing systems increases soil temperature and reduces the effects of cold damage. In our study. the best result was obtained from the low tunnel with antifreeze. We suggest that the use of plant antifreeze can be beneficial to the plants and provide for optimum plant growth. Plant antifreeze can be used to prevent plants from cold damage especially in winter and autumn season. Using antifreeze also increase yield and quality of plants in autumn and winter periods. This study can be a model research for other plants for protecting cold damage therefore more scientific studies need to research about effect of plant antifreeze on vegetables and other plants in the future.

REFERENCES

[1] Anonymous (2016a): http://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=DIYARBAKIR#sfB. – Access date: 05.03.2016.

[2] Anonymous (2016b): http://www.fao.org/3/y5022e/y5022e03.htm.

[3] Aybak, H. Ç. (2002): Salata ve Marul yetiştiriciliği. – Hasad Yayıncılık, İstanbul.

[4] Buchner, O., Steiner, P., Andosch, A., Holzinger, A., Stegner, M., Neuner, G., Lütz-Meindl, U. (2020): A new technical approach for preparing frozen biological samples for electron microscopy. – Plant Methods 7(16): 48.

[5] Çimen, İ., Turgay, B., Pirinç, V. (2010): Effect of solarization and vesicular arbuscular mychorrizal on weed density and yield of lettuce (*Lactuca sativa* L.) in autumn season. – African Journal of Biotechnology 9(24): 3520-3526.

[6] Demir, H., Gölükçü, M., Topuz, A., Özdemir, F., Polat, E., Şahin, H. (2003): Yedikule ve Iceberg tipi marul çeşitlerinin mineral madde içeriği üzerine ekolojik üretimde farklı organik gübre uygulamalarının etkisi. – Akdeniz Üniversitesi Ziraat Fakültesi Dergisi 16(1): 79-85.

[7] Griffith, M., Lumb, C., Wiseman, S. B., Wisniewski, M., Johnson, R. W., Marangoni, A. G. (2005): Antifreeze proteins modify the freezing process in planta. – Plant Physiol. 138(1): 330-40. DOI: 104/pp.104.058628.

[8] Günay, A. (1981): Serler Cilt II. özel sebze yetiştiriciliği. – Çağ Matbaası, Ankara.

[9] Güngör, F., Çukadar, K. (1999): Erzincan koşullarında ikinci ürün olarak sonbahar yetiştiriciliğine uygun turşuluk hıyar ve marul çeşitlerinin belirlenmesi üzerine araştırmalar. – Erzincan 7.

[10] Gupta, R., Deswal, R. (2014): Antifreeze proteins enable plants to survive in freezing conditions. – Journal of Biosci. 39(5): 931-44. DOI: 10.1007/s12038-014-9468-2.

[11] Güvenç, İ., Kaymak, H. Ç., Karataş, A. (2002): Alçak tünelde farklı dikim zamanlarının marulda bitki gelişmesi ve verime etkisi. – Atatürk Üniv. Ziraat Fak. Derg. 35(1-2): 35-38.

[12] Koçar, G. (2001): Farklı renklerde polietilen ile malçlamanın sera marul yetiştiriciliğine etkileri. – Anadolu. J. of Aarı 11(1): 47-55.

[13] Tüzel, Y., Öztekin, G. B., Duyar, H., Eşiyok, D., Kılıç, G. Ö., Anaç, D., Kayıkçıoğlu, H. H. (2011): Organik salata-marul yetiştiriciliğinde agryl örtü ve bazı gübrelerin verim. kalite. yaprak besin madde içeriği ve toprak verimliliği özelliklerine etkileri. – Tarım Bilimleri Dergisi 17: 190-203.

[14] Ünlü, Ö. H., Ünlü, H., Karataş, A., Padem, H., Kitiş, Y. E. (2006): Farklı renkteki malçların domateste verim ve kalite özelliklerine etkisi. – Alatarım 5(1): 10-14.

[15] Jørgensen, S.K.K, Keskin, S., Kitsios, D., Commerou, P.W., Nilsson, B., Vincents, B. (2008). Antifreeze Proteins : The Applications of Antifreeze Proteins in the Food Industries. Roskilde University.