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Mitigating Cold Stress in Radish (*Raphanus sativus* L.) Through Foliar Nutrient Applications.

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ABSTRACT
Cold stress significantly limits the growing season of plants, leading to substantial losses in both crop yield and quality. Due to global climate change, the damage caused by cold stress to agricultural crops is steadily worsening. In the present study, we investigated the effects of foliar applications of calcium and potassium on yield, quality, and plant growth in radish under cold stress conditions. Radish seedlings were exposed to cold stress by planting them at five different times. In the study, foliar applications of 1% K_2SO_4 and $Ca(NO_3)_2$ were evaluated. The results indicated that the highest tuber weight was obtained from the 1% Ca application. Similarly, the best results in tuber diameter and tuber length were also achieved with the 1% Ca treatment. Moreover, the highest shoot length and shoot thickness were observed in the same treatment. The potassium treatment also produced better results compared to the control, similar to the calcium application. However, no significant differences were observed in chlorophyll index, leaf fresh weight, leaf dry weight, and relative water content of the leaves among the treatments. These results indicate that both calcium and potassium play crucial roles in mitigating the negative effects of cold stress, thus promoting better growth and yield in radish. Furthermore, while both treatments provided benefits, the calcium application appeared to be more effective in improving tuber characteristics. These findings suggest that optimizing nutrient management under cold stress conditions could significantly enhance crop productivity. Further research is needed to determine the optimal application doses and timing under varying ecological conditions. In conclusion, foliar applications of 1% K_2SO_4 and $Ca(NO_3)_2$ are recommended for achieving good plant growth and high yield in radish cultivation under cold stress conditions.

Keywords: *Chilling stress, abiotic stress, nutrient uptake, root vegetable, plant growth.*

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INTRODUCTION

Cold stress (low temperature stress) significantly affects both crop yield and fruit quality by disrupting the physiological processes of plants [1, 2, 3]. Low temperature stress disrupts photosynthesis in plants, leading to oxidative stress, which consequently results in cell death [4]. Furthermore, it poses a significant threat by influencing the geographical distribution of plant species [1].

Plants have developed various mechanisms to tolerate low temperature stress. In order to enhance their tolerance to cold stress, plant cells increase the activities of antioxidant enzymes such as peroxidase, superoxide dismutase, ascorbate peroxidase, and catalase; in addition, they accumulate osmotic regulatory compounds such as soluble proteins, proline, and soluble sugars [3]. Plant nutrition practices can further strengthen these defense mechanisms. In particular, nitrogen (N), phosphorus (P), and potassium (K) fertilizers increase the levels of proline, soluble proteins, and antioxidant enzymes in plants under cold stress, thereby reducing the effects of reactive oxygen species (ROS) and preserving the integrity of cell membranes [5].

Potassium regulates intracellular osmotic pressure in plants, thereby lowering the freezing point and protecting plants against cold stress. In addition, it helps maintain cell membrane integrity and fluidity, reduces membrane damage, and supports the antioxidant defense system by limiting the accumulation of reactive oxygen species (ROS). These effects indicate that potassium plays a key role in enhancing cold stress tolerance in plants [6]. Similarly, calcium (Ca^{2+}) triggers intracellular signal transduction under cold stress and enhances the expression of CBF/COR genes through calmodulin (CaM) and CAMTA transcription factors [7]. Moreover, it regulates stress responses and improves cold tolerance by activating the CBL-CIPK and MAPK signaling pathways [8]. Through these signaling pathways, calcium provides an effective molecular defense against low temperatures in plants [7, 8].

Radish (*Raphanus sativus* L.) is a vegetable rich in vitamins and minerals, playing an important role in human nutrition. Cold stress is a major environmental factor that adversely affects growth, yield, and quality in radish plants. Studies have shown that potassium protects plants against cold stress by maintaining osmotic balance and enhancing antioxidant defense, while calcium provides protection by triggering signaling pathways that activate cold-responsive genes [8, 9]. In this study, it was aimed to determine the effects of foliar fertilization with potassium and calcium on agronomic and physiological parameters of radish (*Raphanus sativus* L.) under cold stress conditions.

Materials and Methods

The study was conducted in a farmer's field located in the İdil district of Şırnak province. At the beginning of the experiment, soil samples were taken from a depth of 0-30 cm and analyzed. The soil used in the experiment consisted of 37.1% sand, 28.4% clay, and 34.5% silt, exhibiting a clay-loam texture. The soil pH was measured as 7.13, indicating neutral characteristics. Lime content was 3.41%, and electrical conductivity was 0.01 dS m^{-1}, classifying the soil as non-saline. Organic matter content was low at 1.21%, and total nitrogen was also low at 0.00008 kg/m^2. Available potassium was found to be sufficient at 0.10588 kg/m^2, whereas phosphorus content was very low at 0.00286 kg/m^2. Among the macronutrients, calcium (Ca) and magnesium (Mg) were low, measured at 1.021 mg/kg and 125.9 mg/kg, respectively. Sodium (Na) content was 88.0 mg/kg. Regarding micronutrients, iron (Fe) was low at 0.04 mg/kg, while copper (Cu), zinc (Zn), and manganese (Mn) were below detectable limits (<0.01 mg/kg), indicating deficiency.

Radish seeds were germinated in a separate location adjacent to the experimental site and subsequently transplanted to the field. Seedlings were planted at five different times (October 21 = 1st planting, October 31 = 2nd planting, November 11 = 3rd planting, November 21 = 4th planting, and November 30 = 5th planting) to expose them to cold stress. The experiment was arranged in a randomized complete block design with three replications. Foliar applications of 0% (control), 1% K_2SO_4, and 1% $Ca(NO_3)_2$ were applied twice at 2-week intervals, starting 20 days after seedling transplantation. Applications were performed using a 16-liter charged backpack sprayer (HB Garden Tools Hb 16 Liter). At harvest, samples were taken from leaves and fruits. Samples were collected from four randomly selected plants per replication.

Assessment of Yield

At maturity, the tuber weight of radishes obtained from four randomly selected plants per treatment group was measured using a scale.

Tuber Diameter and Tuber Length

The diameter of five radishes randomly selected from each replication was measured at the equatorial region, while the length was measured from the tip to the base of the petiole using a Stainless Hardened digital caliper (Digital Caliper 0–150 mm).

Shoot Length and Shoot Thickness

In five randomly selected plants from each replication, shoot length was determined as the distance from the base to the tip of the leaf, and shoot thickness was measured at the widest point using the same Stainless Hardened digital caliper (Digital Caliper 0–150 mm).

Chlorophyll Index

Leaf chlorophyll content was measured after treatments on five randomly selected plants per replication using a SPAD chlorophyll meter (Konica Minolta, SPAD-502 Plus, Tokyo, Japan). Measurements were taken at the midsection of individual leaves with the SPAD meter.

Leaf Fresh and Dry Weight

Fully developed leaves collected from five randomly selected plants per replication were weighed to determine fresh leaf weight. The same leaf samples were then dried in a drying oven at 65 ± 2 °C until constant weight was achieved, and dry weights were recorded using a precision scale. These data were used to assess the fresh and dry biomass amounts.

Relative Water Content of Leaves

Samples were taken from fully developed compound leaves located in the middle parts of radish plants randomly selected from each replication. Fresh weights of the leaves were immediately measured using a precision scale after sampling. Then, leaves were placed in beakers containing distilled water and kept at room temperature for 4 hours. After this period, excess surface water was gently removed using filter paper, and turgid weights were measured again with a precision scale. Subsequently, leaf samples were dried in a Mikrolab-Mipro drying oven set at 65 °C until constant weight was reached, and dry weights were recorded. Relative water content of radish leaves was calculated using the fresh, turgid, and dry weight data according to the following formula [10]:

$$\text{RLWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Statistical Analysis

The experiment was established according to a randomized complete block design with three replications, each consisting of 15 plants. The obtained data were subjected to analysis of variance (ANOVA) using JMP 8 statistical software (SAS Institute Inc., Cary, NC, USA). Differences between treatment means were compared using Tukey's test at a significance level of $P < 0.05$.

Results and Discussions

The results of the study revealed statistically significant differences in the effects of treatments on average tuber weight (Table 1; $P < 0.05$). Compared to the control treatment, calcium (Ca) application under cold stress produced the highest average tuber weight. Notably, Ca treatment during the third planting date significantly increased average tuber weight compared to the control group. Similarly, Ca application in the fourth planting date also resulted in higher tuber weights relative to the control.

Potassium (K) treatment showed a tendency to increase average tuber weight as well. These observations indicate that foliar applications of Ca and K under cold stress confer benefits in terms of average tuber weight (Table 1).
Studies investigating the effects of foliar applications of calcium (Ca), potassium (K), and other foliar fertilizers on radish plants under cold stress are limited. However, some research has addressed the impact of foliar fertilization on radish under similar stress conditions. Foliar application of cobalt sulfate ($CoSO_4$) and proline has been reported to mitigate the adverse effects of salinity stress in radish by increasing plant height, root length, fresh and dry weights, enhancing chlorophyll content, and improving antioxidant enzyme activities [11]. Another study found that potassium nitrate (KNO_3) application in salinity-stressed radish (*Raphanus sativus* L.) increased the levels of phenolic compounds, flavonoids, ascorbic acid, and anthocyanins, while also elevating the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) [12].

Table 1. Effects of treatments on average tuber weight (g)

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	37.02 b	32.95	27.15 b	21.90 c	5.34
Ca	86.85 a	52.11	118.61 a	49.81 a	14.40
K	87.31 a	56.51	60.25 b	32.18 b	13.61
Mean	70.39	47.23	68.67	34.63	11.11
P	0.023*	0.150 ^{ns}	0.0050*	0.0003*	0.0560 ^{ns}

ns: not significant
*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).
The effects of the treatments on tuber diameter were found to be significant on the first, second, third, and fourth planting dates (Table 2; P < 0.05). Under cold stress conditions, calcium (Ca) application increased tuber diameter in radish plants. Compared to the control, Ca application resulted in the highest tuber diameter. Notably, Ca application on the third planting date increased tuber diameter by 78.9%, from 32.88 mm in the control group to 58.81 mm. Potassium (K) treatment also led to an increase in tuber diameter compared to the control (Table 2).

Table 2. Effects of treatments on tuber diameter

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	33.66 b	28.90 a	32.88 b	27.64 b	11.30
Ca	48.30 a	50.58 a	58.81 a	40.47 a	17.50
K	47.23 a	36.02 b	41.47 b	28.51 b	16.15
Mean	43.06	38.50	44.38	32.20	14.98
P	0.0140*	0.005*	0.004*	0.030*	0.074 ^{ns}

ns: not significant
*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).
Our results demonstrated that the treatments had statistically significant effects on tuber length (Table 3; P < 0.05). Under cold stress conditions, calcium (Ca) application resulted in higher tuber lengths across all planting dates compared to the control group. On the third planting date, Ca application increased tuber length by 79.7%, from 42.56 mm in the control to 76.48 mm. Similarly, potassium (K) application under cold stress conditions also led to an increase in tuber length (Table 3).

Table 3. Effects of treatments on tuber length

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	54.66 b	43.39 b	42.56 c	48.18	25.05 b
Ca	67.94 a	61.49 a	76.48 a	51.18	45.68 a
K	66.86 a	58.24 a	63.94 b	59.09	41.45 a
Mean	63.15	54.37	60.99	52.81	37.39
P	0.0290*	0.0210*	0.0007*	0.3390 ^{ns}	0.005*

ns: not significant
*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).
Plant nutrition enhances plant resistance against many abiotic stress factors, including cold stress. In general, proper plant nutrition can alleviate the effects of stress by activating mechanisms such as increased photosynthetic activity, activation of stress-related genes, detoxification of reactive oxygen species (ROS), and stabilization of cell membranes [13,14,15]. In particular, essential macronutrients such as potassium protect plants against stress factors by supporting enzymatic activity, maintaining intracellular osmotic balance, and enhancing antioxidant systems [14, 15]. Our results revealed that foliar application of calcium and potassium under cold stress positively affected tuber development in radish plants, including tuber weight, tuber diameter, and tuber length.
Statistically significant differences were detected in the effects of the treatments on shoot length (Table 4; P < 0.05). According to Table 4, the highest shoot length was observed in the Ca treatment. On the third planting date, Ca application

produced longer shoots compared to the control, increasing shoot length from 12 mm to 17 mm. Potassium (K) treatment also tended to increase shoot length, although the effect was not as consistent as that observed with Ca application (Table 4).

Table 4. Effects of treatments on shoot length

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	20.00 a	15.00 a	12.00 b	10.00 b	7.33 b
Ca	21.33 a	19.33 b	17.00 a	13.66 a	11.00 a
K	19.16 a	18.00 b	16.00 a	11.33 ab	8.66 b
Mean	20.16	17.44	15.00	11.66	8.99
P	0.195^{ns}	0.032*	0.005*	0.033*	0.013*

ns: not significant

*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).

According to Table 5, the treatments had statistically significant effects on shoot thickness (Table 5; P < 0.05). Calcium (Ca) application resulted in higher shoot thickness values under cold stress across all planting dates when compared to the control group. Specifically, the Ca treatment on the fourth planting date increased shoot thickness by approximately 69% relative to the control. Potassium (K) application also tended to increase shoot thickness under cold stress, although the increase was not as pronounced as with Ca application. These findings indicate that foliar applications of Ca and K under cold stress have a positive impact on shoot thickness (Table 5).

Table 5. Effects of treatments on shoot thickness

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	3.99 b	2.59 b	3.03 b	2.67 c	2.74 b
Ca	4.99 a	3.18 a	4.20 a	4.52 a	3.34 a
K	3.47 c	3.05 a	4.41 a	3.61 b	3.30 a
Mean	4.15	2.94	3.88	3.60	3.12
P	0.0005*	0.020*	0.004*	0.004*	0.006*

*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).

The effect of foliar applications of calcium (Ca) and potassium (K) fertilizers on chlorophyll index under cold stress was found to be statistically insignificant (Table 6; P < 0.05). Cold stress may have inhibited chlorophyll synthesis by causing ionic imbalance and osmotic stress in plants. Additionally, the soil in the experimental area was found to have low levels of magnesium (Mg) and iron (Fe). The insufficient availability of Mg and Fe, which are directly involved in chlorophyll synthesis, may have further limited chlorophyll formation under the influence of cold stress.

Table 6. Effects of treatments on chlorophyll index

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	33.53	42.26	42.90	37.23	41.23
Ca	35.00	43.16	42.26	39.13	42.23
K	36.96	43.93	47.00	38.73	41.03
Mean	35.16	43.11	44.05	38.36	41.43
P	0.329 ^{ns}	0.783 ^{ns}	0.071 ^{ns}	0.124 ^{ns}	0.178 ^{ns}

ns: not significant

*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).

While Ca and K applications generally led to higher leaf fresh weight compared to the control group across all sowing dates, these improvements were not statistically significant (Table 7; P < 0.05).

Table 7. Effects of treatments on leaf fresh weight

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	56.78	60.34	54.40	56.98	4.44
Ca	60.65	64.56	76.31	68.43	7.01
K	56.55	69.83	72.30	61.14	6.45
Mean	57.99	64.91	67.67	62.18	5.96
P	0.499 ^{ns}	0.159 ^{ns}	0.190 ^{ns}	0.320 ^{ns}	0.094 ^{ns}

ns: not significant

*: Means followed by different letters in the same column are significantly different according to Tukey's test (P < 0.05).

The effect of treatments on leaf dry weight was found to be statistically insignificant, similar to their effect on leaf fresh weight (Table 8; P < 0.05).

A review of the literature reveals a lack of studies investigating the effects of foliar fertilization on leaf fresh and dry weights of radish plants under cold stress conditions. Most existing studies have focused on salinity, drought, and heavy metal stresses.

The results of the present study indicate that foliar applications of calcium (Ca) and potassium (K) under cold stress did not have a statistically significant effect on leaf fresh and dry weights. This may be attributed to the suppression of metabolic activities, cell division, and expansion in radish plants under cold stress, which could have limited nutrient uptake or prevented the foliar fertilizers from exerting their full physiological effects.

Table 8. Effects of treatments on leaf dry weight

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	4.95	5.45	5.32	4.95	4.44
Ca	5.25	6.08	5.68	6.54	7.01
K	4.93	6.99	6.66	5.02	6.45
Mean	5.04	6.17	5.88	5.50	5.96
P	0.926 ^{ns}	0.188 ^{ns}	0.196 ^{ns}	0.362 ^{ns}	0.093 ^{ns}

ns: not significant

Statistically significant differences in leaf relative water content were observed only on the fifth sowing date (Table 9; $P < 0.05$). On this date, the highest relative water content was recorded in the Ca treatment with 100.00%, while the lowest value was observed in the K treatment with 91.55%. In the control group, this value was measured as 93.26%. According to the obtained data, foliar applications of Ca and K under cold stress did not generally have a statistically significant effect on leaf relative water content (Table 9).

Table 9. Effects of treatments on leaf relative water content

Treatment	1st Planting	2nd Planting	3rd Planting	4th Planting	5th Planting
Control	93.20	91.63	93.76	98.09 a	93.26 b
Ca	84.82	89.83	93.12	95.32 a	100.00 a
K	89.87	89.92	94.96	97.12 a	91.55 b
Mean	89.33	90.46	93.94	96.84	94.97
P	0.319 ^{ns}	0.555 ^{ns}	0.630 ^{ns}	0.305 ^{ns}	0.018*

ns: not significant

*: Means followed by different letters in the same column are significantly different according to Tukey's test ($P < 0.05$).

Conclusion

In this study, the effects of foliar applications of 1% calcium nitrate and potassium sulfate on plant morphology and yield parameters of radish (*Raphanus sativus* L.) under cold stress conditions were investigated. In summary, the results demonstrated that foliar application of calcium significantly enhanced yield and growth parameters such as tuber weight, diameter, length, as well as shoot length and thickness. Similarly, potassium application also positively influenced yield and plant development parameters. In contrast, under cold stress, foliar applications of calcium and potassium did not produce statistically significant effects on physiological parameters such as chlorophyll index, leaf fresh and dry weight, or relative water content. Overall, calcium- and potassium-based foliar fertilizers were found to be effective in improving tuber development in radish plants exposed to cold stress, while their effects on physiological responses were limited. Consequently, foliar applications of calcium and potassium may be recommended to support tuber development in radish cultivation under cold stress conditions.

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