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Effect of Smoke Solution Doses on the Germination Performance of Radish Seeds Under Salt Stress

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Abstract: This study aimed to evaluate the effects of different smoke solution doses (0%, 0.1%, 0.5%, and 1%) derived from hazelnut husk on the germination performance and oxidative stress response of two radish (*Raphanus sativus* L.) cultivars under salinity stress (150 mM NaCl). Morphological parameters (root and shoot length, fresh weight, germination percentage, and vigor index) and malondialdehyde (MDA) levels were assessed. The results showed that salt stress markedly increased MDA content, indicating enhanced oxidative damage in both cultivars. However, low smoke solution doses, particularly in the 2nd trial 0.1%, alleviated oxidative stress and improved germination traits in Ateş, while the 0.5% dose promoted root growth and vigor in Akkaya. The findings indicated that salinity stress increased MDA content, reflecting oxidative damage, and negatively affected germination in radish, with cultivar-specific responses. Moreover, smoke solution treatments exhibited dose- and cultivar-dependent effects, some of which may enhance germination performance, while others may induce inhibitory responses in certain physiological parameters.

1. Introduction

Radish (*Raphanus sativus* L.) is a root vegetable belonging to the *Brassicaceae* (*Cruciferae*) family, consumed fresh and rich in nutrients. A good source of nutrients such as vitamin C, folic acid, and potassium, radish also contains B vitamins, magnesium, and calcium (Zohary and Hopf, 2000; Wang and He, 2005). Cultivation of red varieties is particularly widespread in our country, and according to Anonymous (2025) data, approximately 180,000 tons of radishes were produced in Türkiye in 2024. Considering both the nutritional value and production potential of radishes, agricultural research on this species is of significant importance.

With global climate change, abiotic stress factors that negatively affect agricultural production are increasing. Among these stress factors, salinity is one of the major problems (Kılıç et al., 2023), causing yield losses by limiting water and nutrient uptake by plants during both germination and early developmental stages (Çavuşoğlu and Kabar, 2007; Ghosh et al., 2014). Studies aimed at reducing the negative effects of salt stress on germination have reported the effectiveness of various growth regulators, particularly the use of phytohormones such as gibberellic acid and cytokinin (Kabar, 1987; Khan and Ungar, 2001). However, the economic and environmental sustainability of these practices is a matter of debate.

Currently, plant-based smoke treatments are emerging as an eco-friendly and natural alternative. In 1990, it was initially found that smoke was more efficacious than heat in promoting seed germination (De Lange and Boucher, 1990). Research on species like Bruniaceae, Themeda, and Audouinia has established that smoke solutions alleviate seed dormancy and enhance germination (Baxter et al., 1994; Brown and Van Staden, 1997). Karrikins, chemicals present in smoke solutions, stimulate germination by enhancing the seed's responsiveness to light, water, and phytohormones (Flematti et al., 2004; van Staden et al., 2004). These chemicals are often volatile molecules produced by the regulated burning of organic carbon compounds, including lignin and cellulose (Harti et al., 2020). These contents may vary depending on the type of plant burned, the collection time, and the texture of the material. It has been determined that water-soluble volatile compounds, produced by the slow combustion of dry or fresh plant material, promote seed germination in many species by breaking dormancy in various ways. The active substances are reported to form primarily at temperatures between 160–200°C and to evaporate at higher temperatures. When dissolved in water, these compounds not only promote germination but also enhance root formation, seedling growth, and flowering (Brown and Van Staden, 1997).

The type of plant material utilized is crucial for making smoke solutions for use in agriculture. Large quantities of hazelnut husks are generated annually as a result of intensive hazelnut (*Corylus avellana* L.) cultivation in Turkey's Black Sea Region. When incinerated, these wastes pollute the ecosystem and cause considerable nutrient losses (Özenç, 2004). However, a sustainable strategy that integrates waste management with the encouragement of plant development may be provided by the controlled burning of these wastes to create smoke solutions. In this regard, smoke solutions present opportunities from an agricultural and environmental standpoint. Although the effects of smoke solutions on germination and seedling development in many plant species have been investigated in the literature, there are limited studies on the effects of these solutions on radish seeds under salt stress conditions. In this context, the aim of this study was to determine the effects of different doses of smoke solutions obtained from controlled burning of hazelnut husk on the germination performance of radish seeds exposed to salt stress.

2. Material and Methods

This study was carried out in the laboratory of the Department of Horticulture, Faculty of Agriculture, Yozgat Bozok University. Two different radish (*Raphanus sativus*) varieties (Ateş, Akkaya) belonging to Küçük Çiftlik seed farming were used as plant material. To obtain the smoke solution, hazelnut husk waste was burned using a specially developed setup. During the process, 1 kg of hazelnut husk was subjected to combustion, and the resulting smoke was absorbed into 4 liters of distilled water, as described by Basaran et al. (2019).

An *in vitro* salt stress experiment was conducted on radish seeds in two independent trials, each with four replicates consisting of 25 seeds per replicate. A total of 1,600 seeds were used in the experiment, with 800 seeds allocated to each variety, including both control and salt treatments. Radish seeds were soaked in 5 mL of smoke solution at different concentrations for 24 hours in petri dishes. Four concentrations of smoke solution were used: 0% (control), 0.1%, 0.5%, and 1%. Salt stress was induced using a 150 mM NaCl solution, as described by Ulukapı et al. (2020). Germination tests were conducted on 40 × 40 cm blotting papers in accordance with ISTA guidelines (ISTA, 1993). Blotting papers used in the control group were moistened with distilled water, while those in the salt treatment group were saturated with a 150 mM NaCl solution, ensuring uniform moisture without any dry areas. Twenty-five seeds were placed on each blotting paper, which was then folded in half, carefully wrapped, and rolled to maintain seed contact and moisture during the germination

period. The rolls were placed upright in plastic containers with their open ends facing upward. A small amount of solution was added to the bottom of each container to maintain moisture and prevent desiccation. The containers were tightly sealed, placed in an incubator, and maintained in darkness at 20 °C for 14 days. On the 14th day, the filter paper rolls were carefully removed from the incubator, unrolled, and subjected to measurements and MDA (malondialdehyde) analysis.

2.1. Measurements and analyses

- Root and Shoot Length (cm): At the end of the experiment, the lengths of the radicle (root) and hypocotyl (shoot) were measured in centimeters using a ruler.
- Fresh Weight (mg): The fresh weight of the germinated seedlings was determined using a precision balance with an accuracy of 0.0001 g.
- Germination Percentage (%): The number of germinated seeds was counted, and germination percentage was calculated based on the total number of seeds.
- Vigor Index: The vigor index was calculated using the formula: *(Root length + Shoot length) × Germination percentage* (Hu et al., 2005; Aydöner Çoban et al., 2020).

Malondialdehyde (MDA) content was determined following the method described by (Rao and Sresty, 2000). A total of 0.5 grams of fresh tissue was placed in glass tubes and homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenates were centrifuged at 6000 rpm for 5 minutes, and 1 mL of the supernatant was transferred to new glass tubes. Subsequently, 4 mL of 20% TCA containing 0.5% thiobarbituric acid (TBA) was added to each tube. The mixture was incubated in a water bath at 95 °C for 30 minutes, then rapidly cooled in an ice bath.

Absorbance of the samples was measured at 450, 532, and 600 nm using a spectrophotometer. MDA content (µmol g^{-1} FW) was calculated using the following formula:

$$MDA = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

2.2. Statistical analysis of data

Statistical analyses were performed using the SPSS 20.0 software package. Differences between the control and salt treatment groups were evaluated by analysis of variance (ANOVA). Duncan's multiple range test was applied to determine significant differences among group means.

3. Results and Discussion

In the study, some morphological measurements were made on the Akkaya and Ateş varieties, and malondialdehyde (MDA) content, an indicator of damage, was determined.

Statistical evaluation of the results obtained from the first and second trials of the Akkaya variety is presented in Table 1. Across both trials conducted on the radish cultivar 'Akkaya', smoke solution treatments showed varying effects on seedling morphological traits under control and salt stress conditions. Under control conditions, shoot length was the only parameter that showed a significant response to the treatments in the first trial ($p < 0.01$), while no significant differences were detected in the second trial. Despite the lack of statistical significance, the 0.1% and 1% smoke doses frequently resulted in numerically higher shoot lengths and vigor indices, indicating a potential stimulatory effect on aboveground growth.

Under salt stress, root length was significantly affected in both trials ($p < 0.05$). In general, moderate doses of smoke solution-particularly 0.5% and 1% led to better root development and improved vigor compared to the control and higher doses. For instance, the 0.5% dose yielded the highest root length in the second trial, while the 1% dose performed relatively well in both trials for vigor and fresh weight. Conversely, the 3% dose often led to reduced root length and vigor under salt stress, suggesting possible inhibitory effects at higher concentrations.

These findings collectively suggest that smoke solution exhibits a dose-dependent and condition-specific influence on radish seedling development, with moderate concentrations providing the most favorable outcomes, especially under saline conditions.

It has been shown that smoke solutions derived from burning plant materials have an impact on seed germination and early seedling development in some agronomic crops (Özbek et al., 2021; Doğrusöz et al., 2022).

Table 1. Effects of smoke solution doses on morphological characteristics in radish seedlings (cv. Akkaya) under control and salt stress conditions.

	Control					**Salt Stress**				
1. Trial Akkaya	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**
0%	12.257	14.495a	78.67	2106.30	262.30	14.904 a	8.300	71.33	1660.33	209.70
0.1%	11.050	11.481b	80.00	1813.60	259.30	10.240 b	7.176	69.33	1184.13	203.00
0.5%	12.760	13.029a	82.67	2159.40	217.70	12.844 ab	8.052	81.33	1699.60	204.70
1%	11.690	13.668a	94.67	2405.40	246.70	12.009 b	8.108	66.67	1341.60	210.30
	ns	**	**ns**	**ns**	**ns**	*	**ns**	**ns**	**ns**	**ns**
2. Trial Akkaya	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**
0%	**11.620**	**11.954**	**89.33**	**2103.20**	**214.33**	**8.867 ab**	**5.545**	**61.33**	**873.27**	**196.67**
0.1%	**11.579**	**13.834**	**89.33**	**2261.73**	**255.33**	**9.683 a**	**6.771**	**78.67**	**1296.78**	**191.00**
0.5%	**11.508**	**11.302**	**88.00**	**2038.99**	**224.67**	**10.008 a**	**5.623**	**70.67**	**1108.79**	**190.00**
1%	**12.125**	**12.450**	**88.00**	**2158.67**	**208.00**	**7.153 b**	**6.088**	**62.67**	**833.79**	**201.00**
	ns	**ns**	**ns**	**ns**	**ns**	*	**ns**	**ns**	**ns**	**ns**
Average	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**
0%	**11.938**	**13.225**	**84.00**	**2112.21**	**238.33**	**11.885 a**	**6.922**	**66.33**	**1249.66**	**203.00**
0.1%	**11.314**	**12.658**	**84.67**	**2034.52**	**257.33**	**9.962 b**	**6.974**	**74.00**	**1247.04**	**197.00**
0.5%	**12.135**	**12.165**	**85.33**	**2092.18**	**221.33**	**11.426 a**	**6.838**	**76.00**	**1391.44**	**197.30**
1%	**11.906**	**13.059**	**91.33**	**2281.08**	**227.33**	**9.580 b**	**7.098**	**64.67**	**1077.21**	**205.30**
	ns	**ns**	**ns**	**ns**	**ns**	*	**ns**	**ns**	**ns**	**ns**

Different letters next to the means indicate statistically significant differences according to Duncan's multiple range test ($p < 0.05$). ns: non-significant; *: $p < 0.05$; **: $p < 0.01$.

Malondialdehyde (MDA) content, as an indicator of membrane lipid peroxidation, was analyzed to evaluate the effects of smoke solution treatments on oxidative stress. In both trials conducted on the 'Akkaya' cultivar, no statistically significant differences were observed

among smoke solution doses under control conditions ($p>0.05$), indicating that the smoke treatments did not affect MDA accumulation in the absence of salt stress (Figure 1).

In contrast, under salt stress, significant differences in MDA content were recorded among the different smoke doses in both trials ($p<0.001$ in the first trial and $p<0.05$ in the second trial). In both cases, 0.5% and 1% smoke solution doses resulted in a significant increase in MDA levels, suggesting that higher concentrations of smoke may intensify membrane lipid peroxidation under salt stress conditions. Notably, the lowest MDA accumulation was observed at the 0.1% dose, indicating that a low concentration of smoke solution may exert a protective effect by reducing salt-induced oxidative damage. These findings suggest that the smoke solution modulates the oxidative stress response in a dose-dependent manner and may provide protective effects at lower doses under salt stress conditions.

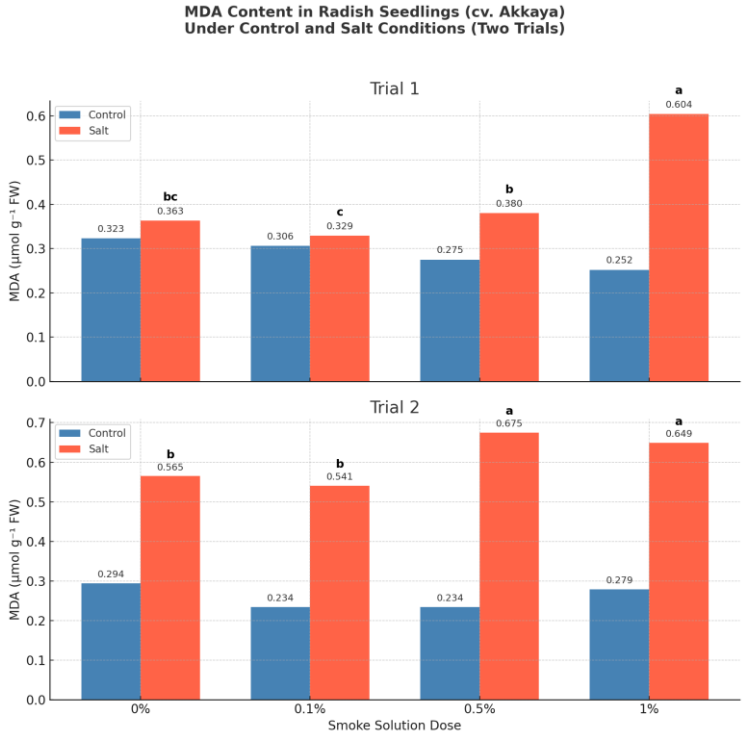

Figure 1. MDA content (µmol g^{-1} FW) in radish seedlings (cv. Akkaya) treated with different smoke solution doses (0%, 0.1%, 0.5%, and 1%) under control and salt stress conditions across two independent trials. Bars represent mean values from each trial. Different letters above bars within the salt treatment indicate statistically significant differences among doses according to Duncan's multiple range test ($p<0.05$).

In the radish cultivar 'Ateş', the effects of smoke solution treatments on seedling morphological traits varied depending on the applied concentration and salt stress condition (Table 2). In the first trial, although no statistically significant differences were observed ($p> 0.05$), the 0.1% smoke solution dose resulted in the highest shoot length (12.482 cm), vigor index (1862.51), and fresh weight under control conditions. However, under salt stress, a notable reduction in germination and vigor was observed at the 1% dose, suggesting a potential inhibitory effect at higher concentrations. In the second trial, smoke solution treatments significantly affected almost all morphological parameters. Under control conditions, the 0% dose produced the highest values for root length (10.888 cm), shoot length (11.923 cm), germination (82.67%), and vigor index (1883.63), indicating no clear benefit from smoke exposure. However, under salt stress, the 1% dose yielded the highest germination (77.33%) and vigor index (1155.07), suggesting that higher smoke

concentrations may offer some protective or stimulating effects under saline conditions. When averaged across both trials, statistically significant differences were found in germination percentage, vigor index, and seedling fresh weight ($p < 0.01$), especially under control conditions. The 0.1% dose consistently showed the highest germination and vigor in non-stressed environments, while moderate doses (0.5% and 1%) were more effective under salt stress. These findings indicate that the response of the 'Ateş' cultivar to smoke treatments is both dose- and condition-dependent, with 0.1% being optimal under normal conditions and 0.5-1% offering moderate benefits under salinity stress. These findings are consistent with earlier studies suggesting that the stimulatory effects of smoke-derived compounds are dependent on dosage (Kamran et al., 2017; Özbek et al., 2021; Doğrusöz et al., 2021). Brown et al. (1993) investigated the effect of smoke produced by burning *Passerina vulgaris* plant material from the fynbos vegetation on seed germination. Among the 32 species tested, germination was promoted by smoke treatment in 25 species, while no significant difference was observed between the smoke-treated and control groups in 3 species. In the remaining 3 species, no germination occurred under either condition.

Table 2. Effects of smoke solution doses on morphological characteristics in radish seedlings (cv. Ateş) under control and salt stress conditions.

	Control					Salt Stress				
1. Trial Ateş	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**
0%	10.209	10.328	72.00	1476.67	209.00	10.103	6.715	60.00	1016.80	232.67
0.1%	10.725	12.482	80.00	1862.51	242.33	11.118	8.649	73.00	1432.33	232.67
0.5%	10.706	12.133	70.67	1623.76	218.00	9.747	7.652	68.00	1186.53	208.33
1%	11.217	12.447	76.00	1804.40	233.67	8.024	8.260	50.67	830.32	224.00
	ns	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
2. Trial Ateş	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**
0%	10.888a	11.923a	82.67a	1883.63a	240.33a	8.079a	5.963 a	53.33 bc	768.93b	210.67
0.1%	9.251 a	10.843a	82.67a	1661.20a	182.33 b	2.896b	3.772 b	34.67 c	217.47c	122.00
0.5%	6.495 b	7.258 b	37.33b	538.80 b	169.33 b	7.358a	5.203ab	64.00ab	802.40b	186.67
1%	7.068 b	9.097ab	52.00b	843.07 b	181.33 b	8.656a	6.308 a	77.33 a	1155.07a	174.00
	******	*****	*******	*******	*****	*******	*****	******	******	**ns**
Average	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**	**Root length (cm)**	**Shoot length (cm)**	**Germ. %**	**Vigor**	**Single Seedling Fresh Weight (mg)**
0%	10.548	11.126	77.33ab	1668.88ab	224.67	9.091	6.339	56.67	885.30	221.67a
0.1%	9.988	11.663	81.33a	1761.90a	212.00	7.007	6.210	54.00	713.73	177.00c
0.5%	8.600	9.695	54.00c	1004.14c	193.67	8.552	6.427	66.00	988.77	197.00bc
1%	9.143	10.772	64.00bc	1282.57bc	207.67	8.340	7.284	64.00	998.24	199.00b
	ns	**ns**	******	******	**ns**	**ns**	**ns**	**ns**	**ns**	******

Different letters next to the means indicate statistically significant differences according to Duncan's multiple range test ($p < 0.05$). ns: non-significant; *: $p < 0.05$; **: $p < 0.01$.

Malondialdehyde (MDA) content was also evaluated in the 'Ateş' radish cultivar to assess membrane lipid peroxidation under different smoke solution doses and salt stress

conditions. Under control conditions, no statistically significant differences in MDA content were observed among the smoke solution treatments in either trial ($p > 0.05$), indicating that smoke alone did not induce oxidative stress (Figure 2). However, under salt stress, significant differences were detected in both trials ($p < 0.05$ and $p < 0.001$, respectively). In the first trial, the 3% dose resulted in the highest MDA level (0.486 µmol g^{-1} FW), significantly different from the 0.1% dose (0.273 µmol g^{-1} FW). Similarly, in the second trial, the 0.1% and 0.0% doses caused significantly higher MDA accumulation (0.574 and 0.613 µmol g^{-1} FW, respectively) compared to 0.5% and 1% doses. This suggests that lower MDA levels were associated with moderate doses of smoke solution (particularly 0.5% and 1%), whereas both untreated and highly treated groups showed elevated oxidative stress. These results support the idea that smoke solution exerts a dose-dependent hormetic effect on oxidative damage, where moderate doses may mitigate stress-induced lipid peroxidation, while very low or high doses fail to provide protection or may even intensify stress under salt conditions. According to the study conducted by Gosh et al. (2014), which investigated the effects of salinity on germination, growth, and yield parameters of radish plants, the impact of salt stress varied depending on the cultivar. Similarly, in the present study, it was observed that the effects of salt stress also differed between cultivars. Munir et al. (2013) examined changes in physiological and biochemical parameters of two different radish cultivars under saline conditions and reported that salt stress significantly reduced shoot fresh and dry weights, with the most pronounced effects observed at a salt concentration of 120 mM.

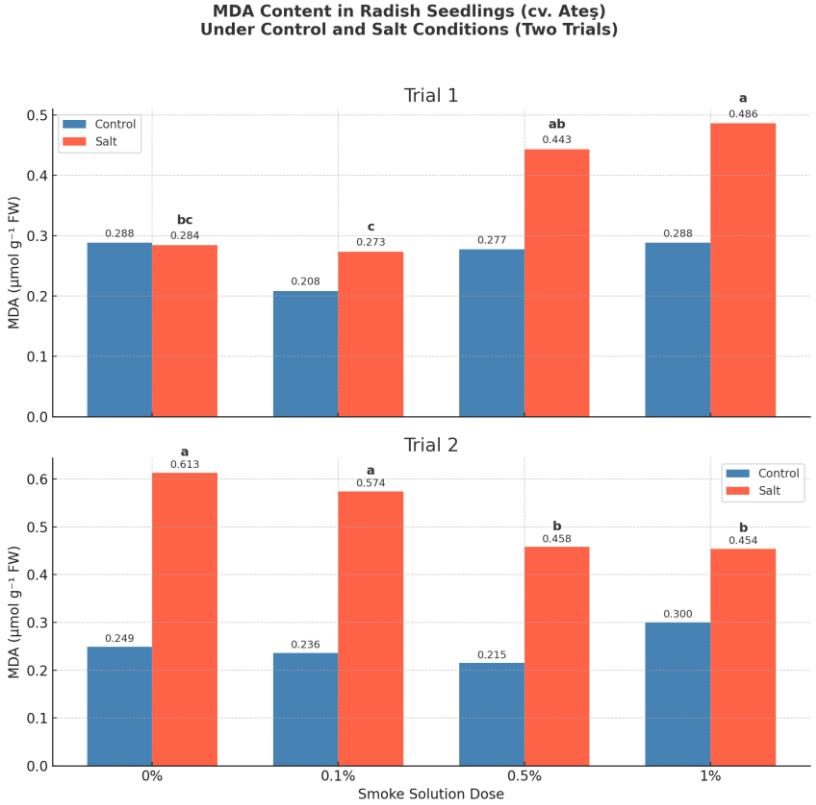

Figure 2. MDA content (µmol g^{-1} FW) in radish seedlings (cv. Ateş) treated with different smoke solution doses (0%, 0.1%, 0.5%, and 1%) under control and salt stress conditions in two independent trials. Bars represent mean values. Different letters above salt treatment bars indicate statistically significant differences among smoke doses according to Duncan's multiple range test ($p < 0.05$ or $p < 0.001$).

Overall, the two radish cultivars exhibited cultivar-specific responses to smoke solution treatments under both control and salt stress conditions. Akkaya showed improved root

growth at moderate smoke doses (0.1–0.5%) under salinity, whereas Ateş responded better to 0.1% smoke dose under non-stressed conditions. These differences highlight the importance of considering cultivar-specific responses when applying smoke solutions to mitigate stress effects.

4. Conclusion

In conclusion, the findings of this study revealed that salinity stress adversely affected both germination and malondialdehyde (MDA) content in radish plants, with the severity of these effects differing among cultivars. The application of smoke solution derived from hazelnut husk exhibited both positive and negative effects depending on the dose and cultivar. Certain concentrations promoted germination and mitigated oxidative stress. In particular, in the second trial, the low smoke solution dose of 0.1% alleviated oxidative stress and improved germination characteristics in Ateş, while the 0.5% dose promoted root growth and vitality in Akkaya. These results suggest that hazelnut husk-derived smoke solutions may offer a promising approach to enhancing salt tolerance in sensitive crops. However, the dose- and cultivar-dependent nature of the responses highlights the need for further research to optimize application concentrations and evaluate long-term effects under varying environmental conditions.

Declaration of Author Contributions

Burak Aktaş was involved in the establishment and execution of the experiment, conducting the analyses, and entering the data. Gökçe Aydöner Çoban contributed to the design and execution of the experiment, performed the analyses, carried out the statistical evaluation of the data, and was responsible for writing the manuscript.

Declaration of Conflicts of Interest

The authors state that there are no conflicts of interest associated with this research.

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