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Growth Media Combination and Penicillium as a Biological Elicitor Reduced the Harmful Effects of Salinity on Strawberry cv. 'Camarosa'

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ABSTRACT

Strawberries are highly sensitive to salinity stress. While elicitors enhance plant defense by stimulating the production of secondary metabolites, zeolite mitigates salinity effects by improving water absorption. This study was conducted using a completely randomized factorial design in a hydroponic greenhouse in Torbat-e Jam, Iran. The experimental treatments included growth media composed of cocopeat, perlite, and zeolite in three ratios: 50:50:0 (control), 50:25:25, and 50:15:35. Fungal elicitors derived from *Penicillium* sp. were applied at concentrations of 0, 4,000, and 8,000 ppm, and salinity levels were set at 0, 20, and 40 mM. Results showed that both *Penicillium* concentration and substrate composition significantly affected enzyme activity, proline content, malondialdehyde (MDA) levels, and shoot fresh and dry weights. MDA levels were lowest in the medium containing 25% zeolite (50% cocopeat + 25% perlite + 25% zeolite) when treated with 8,000 ppm *Penicillium*, indicating reduced oxidative stress and reactive oxygen species. The highest proline content and antioxidant enzyme activity were observed in plants grown in the medium with 35% zeolite (50% cocopeat + 35% zeolite + 15% perlite) without *Penicillium* treatment. However, notable improvements in these parameters occurred with the application of 4,000 ppm *Penicillium*. In conclusion, growth media containing zeolite at 50:25:25 or 50:15:35 ratios of cocopeat, perlite, and zeolite, in combination with 4,000 or 8,000 ppm *Penicillium*, proved effective in alleviating salinity stress in strawberry cultivation.

Abbreviations: Catalase (CAT), Malondialdehyde (MDA), Phenylalanine ammonia-lyase (PAL), Super oxide dismutase (SOD)

Introduction

Strawberries (*Fragaria* spp.) are herbaceous, perennial, and evergreen plants native to European forests. Wild varieties, distinguished by their small flowers and leaves, have been cultivated since the 14th century for medicinal purposes (Hummer, 1995; Bors and Sullivan, 1998). The cultivated strawberry (*Fragaria* × *ananassa* Duch.), a member of the Rosaceae family, is now grown widely across the globe (Darrow, 1966). However, strawberry cultivation faces a major challenge: sensitivity to high electrical conductivity (EC), which varies among cultivars. Research shows that yields begin to decline when EC exceeds 1 dS m^{-1}, with approximately a 33% reduction in yield for every additional dS m^{-1} (Grieve et al., 2012). Salinity, a widespread global issue, impairs plant growth and reduces productivity. In strawberries, salt stress leads to sodium accumulation in roots and shoots, which

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induces leaf necrosis and depletes potassium levels, further compromising plant health (Saidimoradi et al., 2019). To address this, elicitors have emerged as promising tools. These are non-toxic compounds that stimulate plant defense mechanisms against both abiotic and biotic stresses (Chakraborty et al., 2019). One such elicitor is zeolite, a naturally occurring mineral with a tetrahedral framework of silicon surrounded by four oxygen atoms. This structure creates channels and cavities (3–8 angstroms in size) that facilitate cation exchange and selective ion sorption (Garadkar, 2018; Mahvi et al., 2016). Zeolite is valued for its ability to alleviate water and salinity stress, supply nutrients, and enhance overall plant vigor (Polat et al., 2004).

Recent studies underscore the efficacy of zeolite under saline conditions. For instance, zeolite nanoparticles have been shown to improve soil water retention and increase potato yields under salinity stress (Mahmoud et al., 2020). Similarly, biological elicitors, stimuli that promote favorable physiological responses, have been found to boost growth, development, and product quality by enhancing stress tolerance, nutrient uptake, and metabolic efficiency (Garza-Alonso et al., 2022). The effects of salinity on strawberry cultivars have been well documented. In a hydroponic system, increased salinity reduced fruit weight by 26% in the 'Corona' cultivar and by 46% in 'Elsanta,' with corresponding decreases in fruit size (Keutgen and Pawelzik, 2008).

Antioxidant enzymes such as ascorbate peroxidase, superoxide dismutase (SOD), catalase (CAT), and phenylalanine ammonia-lyase (PAL) play crucial roles in detoxifying reactive oxygen species. Among them, SOD is particularly effective in mitigating oxidative damage caused by salt stress and sodium accumulation in plant tissues (Shah et al., 2021). With salinity levels rising in water sources commonly used for greenhouse cultivation, addressing the negative impacts of salt stress has become increasingly important. In this context, the present study aimed to evaluate the potential of *Penicillium* and zeolite-enriched growth media to enhance strawberry tolerance to salinity stress.

Material and methods

Strawberry seedlings (*Fragaria* × *ananassa* cv. 'Camarosa') at the three-leaf stage were used in this study. The seedlings were obtained from the Baghdari Mashhad Company under the supervision of the Ministry of Agriculture Jihad. The experiment was conducted using a completely randomized factorial design with three replications. After four weeks of initial growth, *Penicillium* treatments were applied. Three days later, saline irrigation commenced, consisting of three cycles of saline water application, each followed by a rinse with normal water. The growth medium was composed of cocopeat, perlite, and zeolite in three combinations: 50% cocopeat + 50% perlite (control); 50% cocopeat + 25% perlite + 25% zeolite; and 50% cocopeat + 15% perlite + 35% zeolite. Two 'Camarosa' seedlings were planted per pot. Plants were cultivated in a greenhouse under natural light, with environmental conditions maintained at 25 °C during the day and 18 °C at night, and relative humidity ranging from 50% to 70%. A heating and cooling system regulated the internal climate to ensure consistent growing conditions.

Preparation and measurement of the nutrient solution

Table 1 provides the elemental composition for the specialized nutrient solution used for feeding the strawberry plants, with 120 g of package A and 118 g of package B dissolved in 200 L of water. From the beginning of planting, all pots were watered with a half-strength Hoagland nutrient solution for one week. As the seedlings grew, they were watered with 300 mL of this solution every 3 d.

Preparation and application of Penicillium

The fungal elicitor was extracted from *Penicillium sp.*, following a method described by Farakya et al. (Farakya et al., 2005). The extracted fungal elicitor was stored at 4 °C until use. Four weeks after the seedlings were established and nourished with Hoagland's solution, *Penicillium* treatments at concentrations of 0, 4,000, and 8,000 ppm were sprayed onto the plants for a period of four weeks.

Salinity stress

Salinity treatments were applied two months after seedling establishment and lasted for four weeks. Sodium chloride (NaCl) was added to Hoagland's nutrient solution to achieve salinity concentrations of 0, 20, and 40 mM.

Separation and measurement of samples

Sampling of strawberries occurred eight months after the start of the experiment. To measure the fresh and dry weight of the shoots, the entire aerial portion of the plants was carefully separated. Immediately after harvest, the fresh weight of the shoots was measured using a high-precision digital scale (accuracy: 0.01 g). The samples were then transferred to the laboratory, dried in an oven at 80 °C for 48 h, and weighed again to determine the dry weight. Fruit and leaf samples were stored at -20 °C in a freezer for further analysis (Table 2).

Table 1. Calculations and amounts of elements in Hoagland specialized solution.

	Elements	Package A (%)	Package B (%)
1	Nitrogen	8.07	12.78
2	Phosphorus	2.62	-
3	Potassium	19.32	-
4	Magnesium	4.13	-
5	Boron	0.045	-
6	Manganese	0.052	-
7	Zinc	0.004	-
8	Copper	0.002	-
9	Molybdenum	0.001	-
10	Iron	0.24	-
11	Sulfur	5.54	-
12	Folic acid	-	0.4
13	Calcium	-	19

Table 2. Measured parameters and related methods.

Parameter	Method
Proline	Bates et al. (1973)
MDA	Elabscience (2009)
CAT	Aebi (1984)
SOD	Minami and Yoshikawa (1979)
PAL	Cheng and Breen (1991)

Malondialdehyde (MDA), Catalase (CAT), Super oxide dismutase (SOD), Phenylalanine ammonia-lyase (PAL).

Data analysis

Data were analyzed with SAS software. Mean values were compared via Duncan's multiple range test ($P \leq 0.05$).

Results

Proline

According to Table 3 and Figure 1a, the highest proline concentration (37.69 μmol g^{-1} protein) was observed in plants grown in the C3 medium (50% cocopeat, 15% perlite, 35% zeolite) at 0 ppm elicitor concentration and a salinity level of 40 mM. In contrast, the lowest proline content (11.52 μmol g^{-1} protein) was recorded in plants grown in the C2 medium (50% cocopeat, 25% perlite, 25% zeolite) treated with 8,000 ppm elicitor under non-saline conditions (0 mM). This variation reflects a 69% increase in proline accumulation compared to the control.

MDA

According to Table 3 and Figure 1b, the comparison of mean values for the triple interaction on the MDA, the highest concentration of MDA (20.64 μmol g^{-1} FW) was observed in culture medium C1 (50% perlite and 50% cocopeat) with an elicitor concentration of 0 ppm and a salinity level of 40 mM. Conversely, the lowest MDA (3.40 μmol g^{-1} FW) was recorded in culture medium C2 (50% cocopeat, 25% zeolite, and 25% perlite), with an elicitor concentration of 8,000 ppm and 0 mM salinity (Fig. 1b).

Assessing the defense enzyme activities

CAT

As is clear in Table 3, The comparison of the average triple interaction effect of culture medium, elicitor, and salinity on CAT enzyme activity revealed that the highest CAT concentration (5.44 μg g^{-1} protein) occurred in the C3 growth medium (50% cocopeat, 35% zeolite, and 15% perlite) with an elicitor concentration of 0 ppm and a salinity level of 40 mM. This increase in CAT activity was 56% compared to the control. In contrast, the lowest CAT concentration (2.39 μg g^{-1} protein) was observed in the C2 culture medium (50% cocopeat, 25% zeolite, and 25% perlite) with an elicitor concentration of 8,000 ppm and 0 mM salinity (Fig. 2a).

SOD

According to Figure 2, the interaction between culture medium, elicitor concentration, and salinity level significantly affected superoxide dismutase (SOD) activity. The highest SOD activity (4.02 µg g^{-1} protein) was recorded in plants grown in the C3 medium (50% cocopeat, 35% zeolite, 15% perlite) with 0 ppm elicitor and 40 mM salinity. In contrast, the lowest SOD activity (1.49 µg g^{-1} protein) was observed in the C2 medium (50% cocopeat, 25% zeolite, 25% perlite) with 8,000 ppm elicitor under non-saline conditions (0 mM). This difference represents a 64% increase in SOD activity compared to the control (Fig. 2b).

PAL

According to the comparison of mean values and interactions among culture medium, elicitor, and salinity on PAL activity, the highest enzyme activity (41.09 nmol g^{-1} protein. min^{-1}) was observed in the C2 growth medium (50% cocopeat + 25% zeolite + 25% perlite) with 0 ppm elicitor and 40 mM salinity. In contrast, the lowest PAL activity (18.29 nmol g^{-1} protein. min^{-1}) occurred in the C1 culture medium (50% perlite + 50% cocopeat) combined with 8000 ppm elicitor and 0 mM salinity. This represented a 96% increase in PAL activity compared to the control (Fig. 2c).

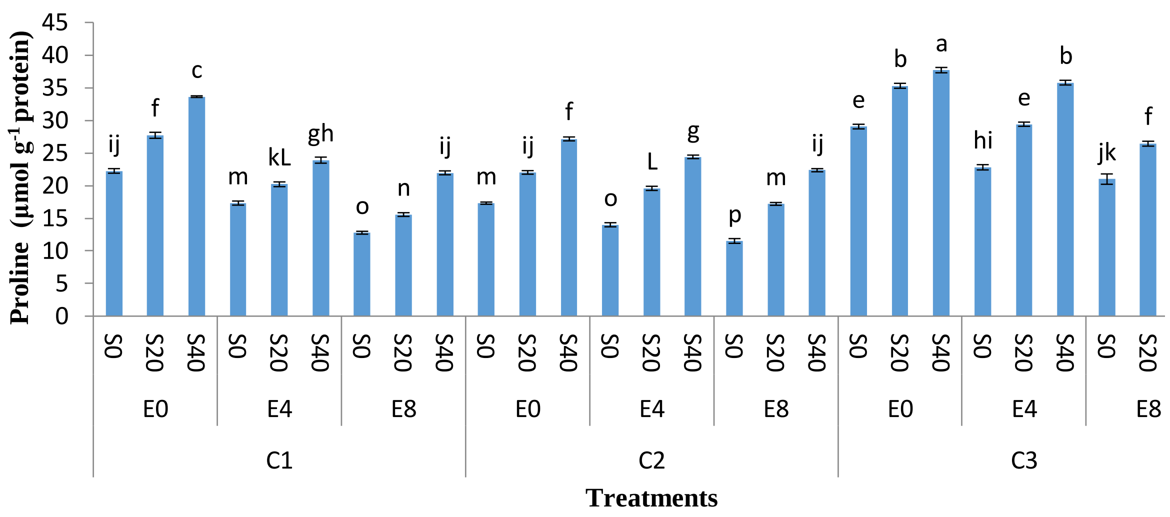
Fresh shoot weight

Analysis of variance indicated that the simple effects of culture medium, elicitor, and salinity on shoot weight were significant ($P \leq 0.01$) (Table 3). Among the culture media, the highest shoot weight (60.28 g) was obtained with the C2 medium (25% zeolite + 25% perlite + 50% cocopeat), whereas the lowest (51.46 g) was recorded in the C1 medium (50% perlite + 50% cocopeat). Switching from C1 to C2 resulted in a 17% increase in shoot weight (Fig. 3a). In terms of elicitor levels, the maximum shoot weight (58.44 g) was observed at 8000 ppm, while the minimum (53.96 g) occurred in the control treatment (0 ppm). Increasing elicitor concentration led to an 8% improvement in fresh shoot weight compared to the control (Fig. 3b). With respect to salinity, shoot fresh weight showed a clear decreasing trend as salinity levels increased. The highest value (64.88 g) was observed at 0 mM salinity, while the lowest (38.48 g) was recorded at 40 mM, reflecting a 25% reduction in shoot fresh weight (Fig. 3c).

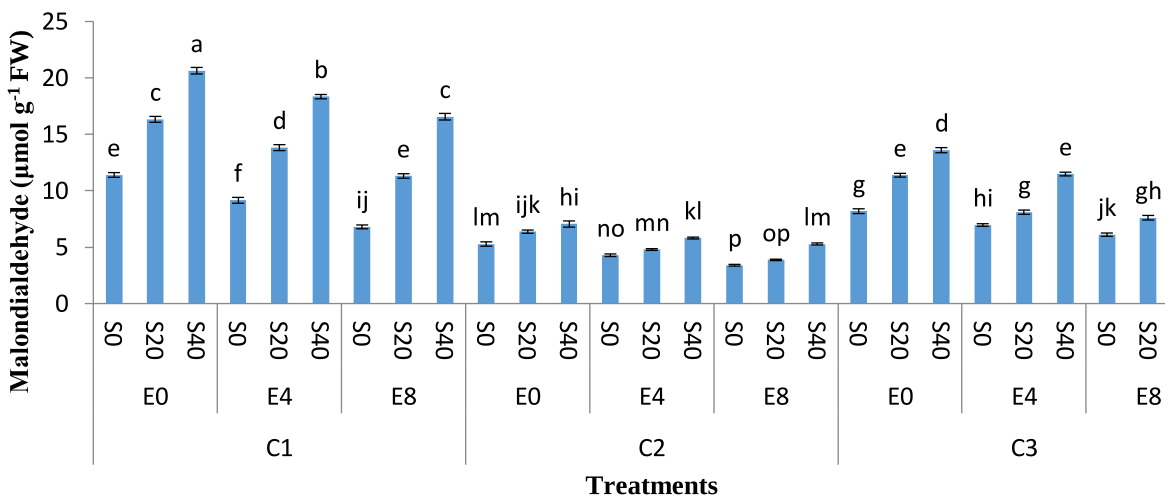
Table 3. Variance analysis of the effects of zeolite and elicitor on some morphophysiological traits of strawberry plants under salt stress.

Source of variation	Degree of freedom	MDA	Proline	CAT	SOD	PAL	Shoot fresh weight	Shoot dry weight
Growth medium	2	510.48**	823.46**	12.73**	13.60**	426.98**	535.09**	19.50**
Elicitor	2	75.94**	428.91**	4.30**	2.12**	393.33**	135.64**	6.60 ns
Salinity	2	179.73**	693.18**	5.67**	3.20**	671.21**	1853.05**	103.03**
Growth medium × Elicitor	4	3.72**	22.21**	0.36**	0.11**	33.99**	5.10 ns	0.10 ns
Growth medium × salinity	4	34.23**	3.78**	0.07**	0.02*	11.28**	22.55 ns	2.62 ns
Elicitor × salinity	4	0.69**	1.08 ns	0.03 ns	0.00 ns	14.31**	13.96 ns	0.28 ns
Elicitor × salinity × growth medium	8	0.66**	4.61**	0.04*	0.02*	5.39*	2.43 ns	0.65 ns
Error	54	0.17	0.59	0.01	0.01	2.25	14.85	1.97
Coefficient of variation (%)		4.35	3.25	3.27	3.32	5.28	6.86	7.03

* and ** respectively indicate significant differences at 0.5 and 1% levels. ns: no differences. Malondialdehyde (MDA), Catalase (CAT), Super oxide dismutase (SOD), Phenylalanine ammonia-lyase (PAL).



(a)

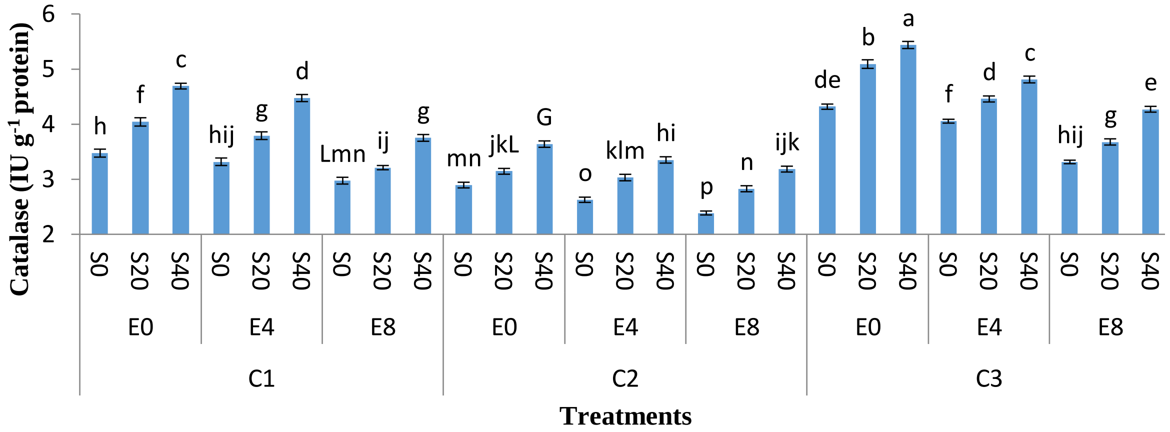


(b)

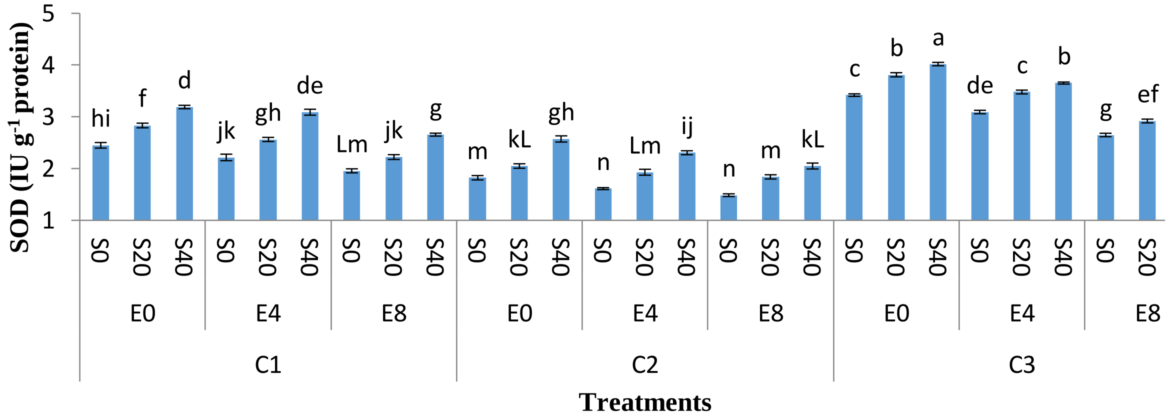
Fig. 1. Comparison of the mean triple interaction effect of growing medium, elicitor, and salinity on proline (**a**); and MDA (**b**) in strawberry. (C_1: 50% by volume of cocopeat and 50% by volume of perlite, C_2: 50% by volume of cocopeat, 25% by volume of perlite, and 25% by volume of zeolite, C_3: 50% of cocopeat, 15% by volume of perlite, and 35% by volume of zeolite, E_0: elicitor at 0 ppm concentration, E_4: elicitor at 4,000 ppm concentration, E_8: elicitor at 8,000 ppm concentration, S_0: 0 mM salinity, S_{20}: 20m M salinity, S_{40}: 40 mM salinity). In each column, mean values that have a common letter are not significantly different based on the LSD test ($P \leq 0.05$).

Shoot dry weight

The comparison of mean values regarding the simple effects of different culture media showed that the highest shoot dry weight (20.80 g) was obtained in the C2 culture medium (25% zeolite + 25% perlite + 50% cocopeat), while the lowest (19.10 g) was recorded in the C1 culture medium (50% perlite + 50% cocopeat). Changing the culture medium from C1 to C2 resulted in a 9% increase in shoot dry weight (Fig. 4a). According to the comparison of mean values, the salinity treatment had a significant effect on shoot dry weight. The highest shoot dry weight (22.10 g) was recorded at 0 mM salinity, while the lowest (18.26 g) occurred at 40 mM salinity. The results showed that increasing salinity led to a 17% decrease in shoot dry weight (Fig. 4b).



(a)



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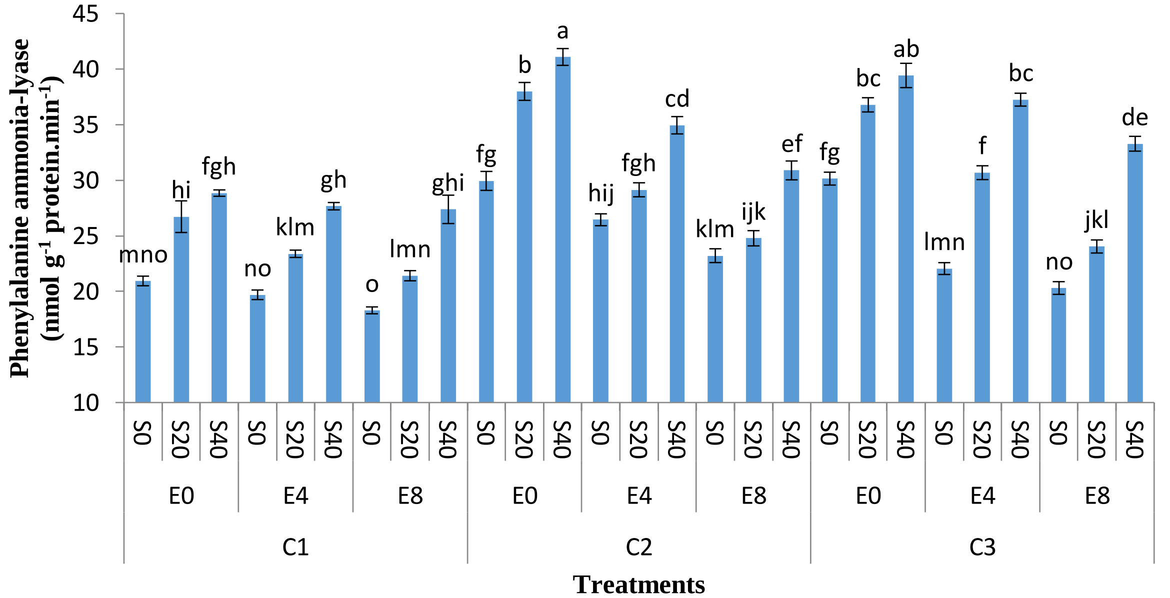

(c)

Fig. 2. Comparison of the mean triple interaction effects of growing medium, elicitor, and salinity on CAT (**a**); SOD (**b**); and PAL (**c**) in strawberry. (C_1: 50% by volume of cocopeat and 50% by volume of perlite, C_2: 50% by volume of cocopeat, 25% by volume of perlite, and 25% by volume of zeolite, C_3: 50% of cocopeat, 15% by volume of perlite, and 35% by volume of zeolite, E_0: elicitor at 0 ppm concentration, E_4: elicitor at 4,000 ppm concentration, E_8: elicitor at 8,000 ppm concentration, S_0: 0 mM salinity, S_{20}: 20 mM salinity, S_{40}: 40 mM salinity). In each column, mean values that have a common letter are not significantly different based on the LSD test ($P \leq 0.05$).

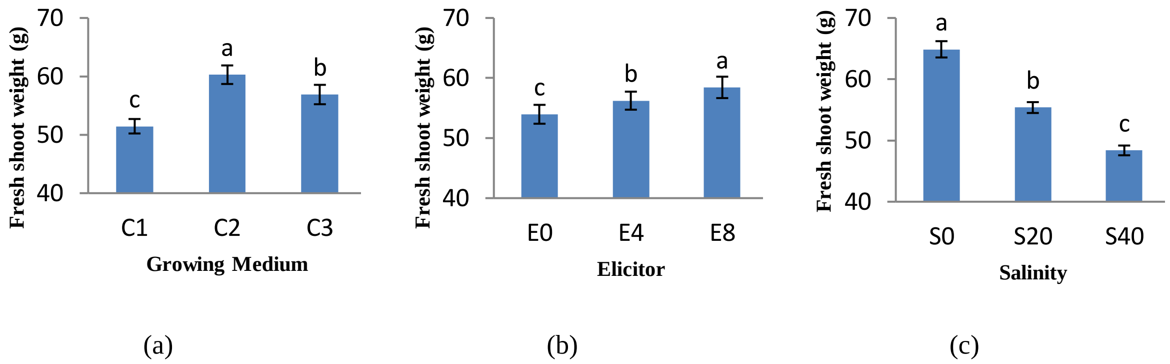

Fig. 3. Comparison of mean values for fresh shoot weight of strawberry in various culture media (**a**); in various elicitor content (**b**); and in various salinity levels (**c**). (C_1: 50% by volume of cocopeat and 50% by volume of perlite, C_2: 50% by volume of cocopeat, 25% by volume of perlite, and 25% by volume of zeolite, C_3: 50% of cocopeat, 15% by volume of perlite, and 35% by volume of zeolite, E_0: elicitor at 0 ppm concentration, E_4: elicitor at 4,000 ppm concentration, E_8: elicitor at 8,000 ppm concentration, S_0: 0 mM salinity, S_{20}: 20 mM salinity, S_{40}: 40 mM salinity). In each column, mean values with a common letter are not significantly different based on the LSD test ($P \leq 0.05$).

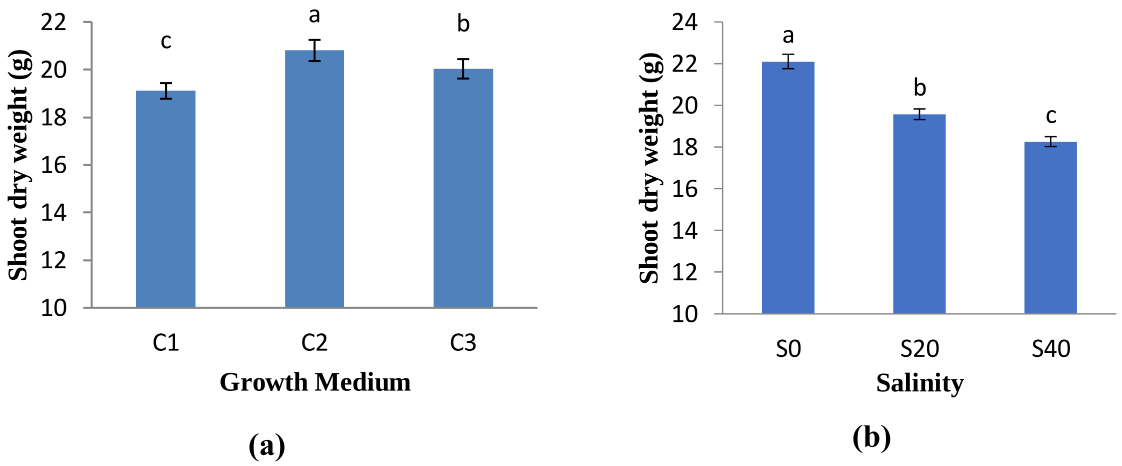

Fig. 4. Comparison of mean values for growth media (**a**); salinity level (**b**) on the shoot dry weight of strawberry. (C_1: 50% by volume of cocopeat and 50% by volume of perlite, C_2: 50% by volume of cocopeat, 25% by volume of perlite, and 25% by volume of zeolite, C_3: 50% of cocopeat, 15% by volume of perlite, and 35% by volume of zeolite, S_0: 0 mM salinity, S_{20}: 20 mM salinity, S_{40}: 40 mM salinity). In each column, mean values with a common letter are not significantly different based on the LSD test ($P \leq 0.05$).

Discussion

Proline plays a crucial role in helping plants cope with stress and is considered highly beneficial in plant tissue culture, where it supports healthier growth under adverse conditions (Pazuki et al., 2015). In the present study, proline levels increased with salinity, particularly in the culture medium containing 35% zeolite by volume, combined with an elicitor concentration of 4,000 ppm. Both the use of elicitors and the composition of the growth medium significantly influenced proline accumulation in strawberry plants under salinity stress, consistent with previous reports (Mahmoud and Swaefy, 2020; Masoudniaragh et al., 2021).

Rhizobacteria treatments also alleviated the negative effects of salinity, leading to substantial increases in leaf area, protein content, and the activities of catalase (CAT) and superoxide dismutase (SOD) in strawberry plants. These treatments significantly reduced leaf sodium content and malondialdehyde (MDA) levels as well (Arikan et al., 2020). Functioning as a key osmolyte and antioxidant, proline contributes to osmotic protection by regulating intracellular osmosis, detoxifying reactive oxygen species (ROS), and stabilizing membrane structures without disrupting cellular metabolism (Balasubramaniam et al., 2023).

Similarly, chitosan elicitors have been shown to mitigate salinity stress in wheat and corn by increasing proline content, thereby supporting osmotic regulation in plant cells (Peykani and Farzami Sepehr, 2018). In another study, the effects of zeolite, compost, and effective microorganisms on apple trees under both normal and saline conditions were investigated. Plants treated with zeolite and effective microorganisms exhibited the highest leaf dry weights across conditions. These treatments also significantly increased proline concentrations in leaves exposed to salinity stress, with zeolite enhancing antioxidant capacity and effective microorganisms supporting stable plant growth (Koulympoudi et al., 2023). Zeolite, a porous aluminosilicate with a negative charge, is known for its high cation exchange capacity (Zandavifard, 2017) and fine pore structure (Polat et al., 2004), which contribute to its effectiveness in agricultural applications. This mineral helps mitigate the adverse effects of salinity stress primarily by enhancing soil water retention. Salinity disrupts essential physiological processes such as water and nutrient uptake and transport, ultimately inhibiting plant growth (Bybordi, 2016). Zeolite’s water-retention properties enable the gradual release of moisture and soluble nutrients, ensuring their continued availability to plants even under moisture stress (Ahmadi Azar et al., 2015). These attributes make zeolite a valuable amendment for improving plant resilience under saline conditions.

An important aspect of plant stress response is the activation of antioxidant defense systems. Salt stress can impair both enzymatic and non-enzymatic antioxidant activities, weakening a plant's defense mechanisms. Among the enzymatic antioxidants, superoxide dismutase (SOD) serves as the first line of defense by converting superoxide radicals into oxygen and hydrogen peroxide, thereby limiting the formation of harmful hydroxyl radicals (Balasubramaniam et al., 2023). Severe salt stress can also suppress protein synthesis; however, the application of elicitors—at low concentrations—can act as controlled stress inducers, triggering defense responses such as the activation of antioxidant enzymes and upregulation of stress-related genes, as demonstrated in *Catharanthus roseus* (Hassan et al., 2021). In the present study, SOD and catalase (CAT) levels increased with rising salinity. Notably, the culture medium containing 35% zeolite and 4,000 ppm elicitor resulted in significant enhancement of both enzymes. Furthermore, the combined application of zeolite and selenium has been reported to significantly improve leaf relative water content, photosynthetic performance, and antioxidant enzyme activity, as supported by earlier studies on the effects of selenium and silicon under stress conditions (Bybordi, 2016).

Phenylalanine ammonia-lyase (PAL) is a key enzyme in the regulation and initiation of the phenylpropanoid pathway, catalyzing the conversion of phenylalanine into trans-cinnamic acid. This pathway is essential for the biosynthesis of a wide range of secondary metabolites, including flavonoids, lignin, tannins, and other phenolic compounds (Ziaei et al., 2012). Under salinity stress, selenium has been shown to act as an antioxidant in garlic plants by increasing PAL activity, thereby enhancing the production of phenols and phenolic compounds as part of the plant’s defense response (Astaneh et al., 2018). Similarly, elicitors are known to boost the accumulation of secondary metabolites, including phenolic compounds and flavonoids, as reported in recent studies (Balusamy et al., 2022; Pandey et al., 2022).

The beneficial effects of zeolite under drought stress have been demonstrated in several crops, including pumpkin (Naeemi et al., 2012), sunflower (Gholam Hoseini et al., 2013), and mint (Ghanbari et al., 2013). In the present study, salinity stress significantly increased PAL activity, reflecting the plant’s biochemical adaptation to stress. Culture media supplemented with 25% and 35% zeolite by volume, in combination with elicitor concentrations of 4,000 and 8,000 ppm, resulted in a marked increase in PAL activity. Salinity stress is also associated with elevated malondialdehyde (MDA) levels, a common marker of lipid peroxidation, particularly in salt-sensitive cultivars. Maintenance of membrane lipid integrity is crucial for salinity tolerance (Mansour, 1998). In this study, increased salinity levels corresponded with higher MDA concentrations and greater cytoplasmic membrane degradation. However, the culture medium containing 25% zeolite and 8,000 ppm elicitor significantly reduced MDA levels, indicating enhanced membrane stability under salinity stress.

These findings align with previous studies demonstrating the protective effects of zeolite and elicitors under abiotic stress conditions. For example, Pizarro et al. (2021) reported reduced MDA content and improved membrane stability in tomato plants treated with zeolite. Similarly, the combined application of zeolite and salicylic acid improved the stress adaptation of wheat plants (Sedaghat et al., 2022). The reduction in MDA levels observed with chitosan elicitor treatments in wheat and corn seedlings has been attributed to its role in minimizing lipid degradation (Shams Peykani and Farzami Sepehr, 2018).

Elicitors are widely recognized for their ability to enhance plant defense mechanisms and resistance, even in the absence of environmental stress, as plants perceive these compounds as signals that trigger protective responses (Iula et al., 2022). Salinity stress has been shown to reduce both dry and fresh weights of shoots and roots in basil plants. However, it simultaneously increases the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT). The application of silicon can alleviate these adverse effects by further enhancing CAT and SOD activity, thereby improving shoot and root biomass under salinity stress (Sharifian Jazi et al., 2023).

Shoots and leaves are essential for energy production and are critical to overall plant growth and development (Ren et al., 2014). Studies in tomato plants have demonstrated that elicitor application promotes the synthesis of fatty acids and secondary metabolites, thereby enhancing resistance to stress and supporting vegetative growth (Iula et al., 2022). Despite this, salinity stress negatively affects lateral stem expansion due to osmotic imbalances around the root zone, which impair water uptake (Balasubramaniam et al., 2023). In the present study, the lowest shoot dry weight was recorded under 40 mM salinity, reflecting the plant's limited tolerance to high salinity levels. This observation is consistent with the findings of Ramdan et al. (2019) who reported that salinity levels exceeding a plant's tolerance threshold significantly reduce both fresh and dry weights of stems and leaves in crops such as sunflower.

Nevertheless, the present study demonstrated that a growing medium composed of 50% cocopeat, 25% perlite, and 25% zeolite significantly improved both fresh and dry shoot weights, indicating its effectiveness in mitigating the adverse effects of water deficiency stress. These findings are consistent with those of Karami et al. (2020), who reported the beneficial impact of zeolite-containing media in alleviating stress-induced growth reductions. Zeolite's ability to enhance water retention positively influences plant growth, as previously observed in rapeseed (Zahedi et al., 2011). However, studies have shown that at lower zeolite concentrations, fresh and dry weights of aerial parts decrease markedly as soil moisture declines (Ahmadi Azar et al., 2015). This suggests that increasing the proportion of zeolite in the substrate improves water retention, allowing water and dissolved nutrients to be gradually released and made available to the plant during moisture stress (Naeimi, 2013).

Moreover, the use of zeolite alone has been shown to maintain leaf area and leaf number in *Aloe vera* under saline conditions (Hazrati et al., 2017), further supporting its role in stress mitigation. In the present study, the highest shoot fresh weight was observed with the application of 8,000 ppm elicitor. Similar results have been reported in *Pueraria tuberosa*, where biological elicitors applied to aerial parts significantly increased shoot number, fresh weight, and dry weight under in vitro conditions. Various biological elicitors, such as yeast extract and pectin, have been shown to enhance cellular metabolism, stimulate antioxidant activity, and promote the biosynthesis of bioactive compounds (Kanthaliya et al., 2023).

Elicitors play a critical role in mediating interactions between plants and their environment by regulating the expression of stress-responsive genes and promoting the synthesis of secondary metabolites (Hassan et al., 2021). In addition to enhancing stress tolerance, elicitors have also been reported to stimulate plant growth and development (Garcia Enciso et al., 2018). These findings collectively highlight the potential of elicitors to enhance plant resilience and productivity under both biotic and abiotic stress conditions.

Conclusions

The results of this study indicated that salinity stress, induced by sodium chloride at a concentration of 40 mM, significantly suppressed vegetative growth traits while enhancing the activity of antioxidant enzymes and proline accumulation in strawberry plants. Specifically, catalase (CAT) and superoxide dismutase (SOD) activities, along with proline content, increased by 56%, 64%, and 69%, respectively, in the C3E0S40 treatment compared to the control. Moreover, the combined application of an elicitor and a zeolite-containing growth medium exerted a significant positive effect on strawberry performance under salinity stress. The highest vegetative growth was recorded in the medium composed of 50% cocopeat, 25% perlite, and 25% zeolite, supplemented with *Penicillium* at 8,000 ppm. It is likely that the inclusion of zeolite improved water and nutrient availability by enhancing retention and gradual release, while also acting as a cation exchanger capable of absorbing excess sodium ions, thereby alleviating salinity-induced toxicity. Additionally, the application of *Penicillium* may have activated the expression of defense-related genes, functioning similarly to a plant immunization response. Together, these factors likely contributed to mitigating the detrimental effects of salinity, promoting the biosynthesis of secondary metabolites, and enhancing antioxidant enzyme activity, ultimately leading to improved vegetative growth. This integrated approach, combining zeolite-based substrates with biological elicitors, presents a promising strategy for improving strawberry cultivation in regions where irrigation with moderately saline water is a necessity.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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