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Research Article

EFFICACY OF SALICYLIC ACID ON MORPHOLOGICAL AND QUALITY PARAMETERS OF STRAWBERRY CV. CHANDLER FRUIT UNDER SALINITY CONDITIONS IN GREENHOUSE

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Abstract

Strawberries belong to the Rosaceae family and are mostly grown in subtropical and temperate regions. Salinity is a hindrance to the potential yield of strawberries in Pakistan. The current experiment was designed to evaluate the impact of salicylic acid (SA) on reducing salinity stress in the strawberry cv. "Chandler. In this experiment, four levels of salicylic acid (SA) concentrations (0, 0.25, 0.50, 1.0mM) as well as four levels of NaCl (0, 60, 90, 120mM) were applied before flowering. The morphological parameters, including vegetative, reproductive and quality aspects, were examined. The treatment combination with 1mM SA and 90mM NaCl showed the best performance. Experimental results show that the morphological parameters, including plant height, leaf area, crown size, canopy spread, and fresh and dry plant weight, as well as the average number of trusses, flowers, and fruit set proportion, fruit yield, and sugar content, increase with an increase in salicylic acid concentration. The experimental result showed that the application of 1mM SA combined with 90mM NaCl for strawberry plants can effectively reduce salinity stress and improve the vegetative growth, fruit yield, and quality.

Keywords: *Salicylic acid, Salinity stress, Strawberry, Greenhouse.*

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1. INTRODUCTION

Strawberries belong to the *Rosaceae* family, originate from North America and France (Amil-Ruiz *et al*., 2011). It is globally popular for its taste, red color, and fragrance. These fruits provide essential vitamins, health-enhancing phytochemicals and minerals along with their unique taste (da Silva *et al*., 2007). The fruit has high vitamin C along with carbohydrates, dietary fiber, lipids, proteins, ascorbic acid, calcium, and vitamin B6 content (Basu *et al*., 2014; Afrin *et al*., 2016). Human consumed it as a fruit as well as its different products, including jams, nectars and juices, which are in commercial usage (Basu *et al*., 2014). The Romans used strawberries for medicinal purposes since the 13th century (Rajwana *et al*., 2016). The difference in experimental results shows that the strawberry fruit has an impact on minimizing the chances of chronic diseases like cancer and cardiovascular ailments

(Afrin *et al*., 2016). Polyphenols and vitamins are vital for mitigating oxidative stress, particularly in relation to neurodegenerative conditions and ageing (Giampieri *et al*., 2018). Strawberries have been found to contain more than 40 phenolic compounds, such as glycosides of quercetin, kaempferol, cyanidin, pelargonidin, ellagitannins, and ellagic acid. The fruit's antioxidant potential is thought to be primarily influenced by ascorbic acid, ellagitannins, and anthocyanins. (Aaby *et al*., 2007). In 2017, the amount of strawberries produced worldwide increased by 5% to 9.22 million tonnes (FAO, 2017). With 40.34% of the world's total output, China leads the pack, followed by the United States (15.61%), Mexico (7.04%), Turkey (4.33%), and Spain (3.9%) (FAOSTAT, 2017). Both temperate and subtropical regions of Pakistan cultivate strawberries as an exotic fruit crop. In the early 1980s, this crop was first grown experimentally in the Khyber Pakhtunkhwa province's districts of Mansehra, Mardan, and Swat. Chandler, Corona, Douglas, Tufts, Gorella, and Toro are among the cultivars that are successfully grown today. With an average production of five to ten tons per hectare, strawberries are grown on 171 hectares, yielding approximately 591 tons annually. Pakistan's strawberry crop has nearly doubled during the past five years. (Rajwana *et al*., 2016). Pakistan, especially Khyber Pakhtunkhwa, has a lot of promise for strawberry farming because the fruit can be used to make other products, processed, and exported. (Tariq *et al*., 2018). Still, results aren't always the same; they range from 3,000 to 5,000 kg per farm (Tariq *et al*., 2018). Pakistan's production is still very low compared to world winners like China (3.72 million tons), the USA (1.44 million tons), and Mexico (0.65 million tons) (Rajwana *et al*., 2016). Inadequate crop management and socio-economic factors such as farmer education and experience are the two major reasons for low yield (Khan *et al*., 2003; Haq *et al*., 2024). The yield of strawberries is also affected by different stresses (Zhu, 2016). Abiotic stresses like salinity, drought, cold, heat, and nutrient differences, as well as biotic stresses like pests and viruses, commonly affect plants' health and hinder their growth (Rejeb *et al*., 2018). Flood irrigation with bad-quality water increases the salinity issue in Pakistan, resulting in a decrease in agricultural productivity on an area of 4.5 mha (Qureshi and Perry, 2021). It damages crops by disturbing water absorption, causing oxidative stress, creating ion toxicity, which results in decreased photosynthesis, insufficient nutrient uptake and stunted growth (Rahimi & Biglarifard, 2011). Strawberries are famous for their extreme salt tolerance, though these levels vary among varieties (Asghar *et al*., 2011). Salinity stress causes accelerated leaf senescence, leaf necrosis, and reduces photosynthetic capacity; it also limits the quantity of carbohydrates available for fruit growth (Saied *et al*., 2005). However, nothing is known regarding the circulation of nutrients and fruit yield cultivated in salty conditions (Keutgen and Pawelzik, 2009). Effective mitigating strategies by applying Salicylic acid, as it consists of a natural phenolic compound famous for increasing salt tolerance in plants. It also provides systemic acquired resistance (SAR) to infections, alters several biochemical systems and physiological processes (Shakirova *et al*., 2003). The application of salicylic acid has shown significant results in mitigating heavy metals and salt stress by promoting plant growth, maintaining stomatal function, and increasing photosynthesis (Singh & Gautam, 2013; Khodary, 2004; Arfan *et al*., 2007). It is also essential for the regulation of antioxidants by controlling reactive oxygen species (ROS) levels through catalase inhibition (Szepesi, 2005).

2. MATERIALS AND METHODS

2.1 Experimental Location and Design

The experiment was conducted during 2018-19 in the field of the Horticulture department at Pir Mehr Ali Shah Arid Agriculture University in Rawalpindi, Pakistan, with (Latitude: 33°N, Longitude: 73°E). The 'Chandler' cultivar of strawberries was obtained from a commercial strawberry farm located in Swat, Khyber Pakhtunkhwa. The plants were grown in plastic bags in a greenhouse using a growing medium that was equal parts soil, sand, and farmyard manure (FYM) (1:1:1). To encourage consistent growth and ideal root development, each bag was placed in the center. A Completely Randomized Design (CRD) with 16 treatment combinations and three repeats was the experimental design used. There were nine plants in each treatment. Thirty days after planting, NaCl at doses of 0 (control), 60, 90, and 120 mM was used to create salinity stress. Salicylic acid (SA) was applied topically at concentrations of 0 (control), 0.25, 0.50, and 1.00 mM 45 days after planting, before the reproductive stage. **Table 1** provides specifics on the therapy combinations.

NaCl (mM)	**SA 0.00 mM**	**SA 0.25 mM**	**SA 0.50 mM**	**SA 1.00 mM**
0	S1N1	S2N1	S3N1	S4N1
60	S1N2	S2N2	S3N2	S4N2
90	S1N3	S2N3	S3N3	S4N3
120	S1N4	S2N4	S3N4	S4N4

2.2. Preparation of Treatment Solutions

2.2.1. Salicylic Acid (SA):

For better solubility, salicylic acid was first dissolved in ethanol and then diluted with distilled water to create final concentrations of 0, 0.25, 0.50, and 1.00 mM in a liter.

2.2.2. Saline Solution (NaCl):

NaCl was dissolved in distilled water to produce concentrations of 0, 60, 90, and 120 mM, which corresponded to electrical conductivity (EC) levels of 0.8, 4.5, 6.5, and 8.5 dS m^{-1}, respectively, for the salinity treatments. A nutrient solution (Grow More 20-20-20 NPK), a completely water-soluble fertilizer intended to promote plant development in saltwater environments, was mixed with these solutions.

2.2.3. Data Collection and Observations:

The data for plant growth and yield, fruit quality, reproductive development, and vegetative growth were gathered.

2.3. Vegetative Growth Parameters

2.3.1. Plant Height (cm):

This parameter was measured from the base of the crown to the topmost photosynthetic tissue with the help of a graduated scale.

2.3.2. Canopy Spread (cm):

Horizontal measurements were completed with a measuring tape to evaluate lateral growth.

2.3.3. Number of Leaves:

The number of leaves for each plant's total was counted, and mean values were calculated.

2.3.4. Number of Trusses:

The number of trusses on each plant was tallied and averaged to assess floral initiation.

2.3.5. Leaf Area (cm^2):

Twenty randomly chosen leaves from each treatment were measured using an ADC leaf area meter.

2.3.6. Fresh and Dry Weight (g):

A precision electronic balance was used to weigh whole plants while they were still fresh, after which they were oven-dried to a constant weight.

2.4. Reproductive and Yield Parameters

2.4.1. Number of Flowers:

Each plant was counted from the first to the last bloom, and the average for each treatment was determined. Number of Fruits:

To assess yield, the total number of fruits produced by each plant was noted.

(1)

$$Fruit\ set\ percentage\ (\%) = \frac{Total\ no.of\ fruit\ set}{Total\ number\ of\ flowers} \times 100$$

2.4.2. Fruit Size (cm):

A digital Vernier caliper was used to measure the diameter and length of six randomly chosen fruits for each treatment.

2.4.3. Weight of Fruit (g):

A Setra BL-410 S balance was used to determine the average weight of six randomly chosen fruits for each treatment.

2.5. Fruit Quality Analysis

Ascorbic Acid (mg/100 ml juice): The Hans (1992) technique was used to calculate the vitamin C content. A spectrophotometer (OPTMA SP-3000-Plus) was used to measure the supernatant at 243 nm after five grams of fruit pulp were homogenized in 0.1% HCl and centrifuged for ten minutes at 10,000 rpm. Total Soluble Solids (°Brix): A portable refractometer was used to measure this at room temperature. The °Brix value was measured after placing a drop of juice on the prism. Total Sugar Content (%): This was calculated using Horwitz's (1960) approach. First, reducing sugars were measured, and then non-reducing sugars were hydrolyzed with HCl. After neutralization with 0.1N NaOH, the solution was titrated with Fehling's solution, and the total sugar was calculated using:

$$Total\ sugar\ (\%) = 25\ x\ \frac{X}{Y} \qquad (2)$$

(Basu *et al*., 2014)

where X = volume used, Y = titration value. Titratable Acidity (%): Ten milliliters of diluted juice were titrated against 0.1N NaOH using phenolphthalein as an indicator. Acidity was calculated as:

$$\text{Titratable Acidity (\%)} = \frac{m \times 0.0064}{10 \times 100} \qquad (3)$$

Where m indicates the used volume of NaOH

2.6. Statistical Analysis

All experimental data were subjected to analysis of variance (ANOVA) using the software **STATISTIX 8.1.** Treatment means were compared using the Least Significant Difference (LSD) test at a significance level of $\mathbf{P \leq 0.05}$, to determine statistically significant differences among treatment effects (Khan *et al*., 2025).

3. RESULTS AND DISCUSSION

Four levels of salicylic acid concentrations (SA: 0.00, 0.25, 0.50, and 1.00 mM) and increasing salinity stress with (NaCl: 0, 60, 90, and 120 mM) were applied to the plants. Average leaf area (cm²), fresh weight (g), and dried weight (g) for each treatment are shown in the table. Growth attributes under salinity were greatly enhanced by the administration of salicylic acid, with 0.50-1.00 mM SA shown to be the most beneficial across intermediate salt levels.

The experimental results showed the significance of salicylic acid (SA) usage in reducing salinity-induced stress in strawberries (Fragaria×ananassa) cv. "Chandler," principally at reasonable salinity levels (90 mM NaCl). Several vegetative and reproductive features were significantly increased by the application of 1.00 mM SA, suggesting that SA functions as an efficient biochemical modulator in stress physiology. Most commonly, osmotic imbalance, oxidative stress and ion toxicity that negatively affect photosynthesis, absorptive and cellular metabolism, slow down the growth of plants in saline conditions.

The increase in the plant height, area of the leaf, the canopy coverage, fresh and dry biomass following the treatment with salicylic acid in this study lead to the fact that it has to do with the salicylic acid.

(a) (b) (c)

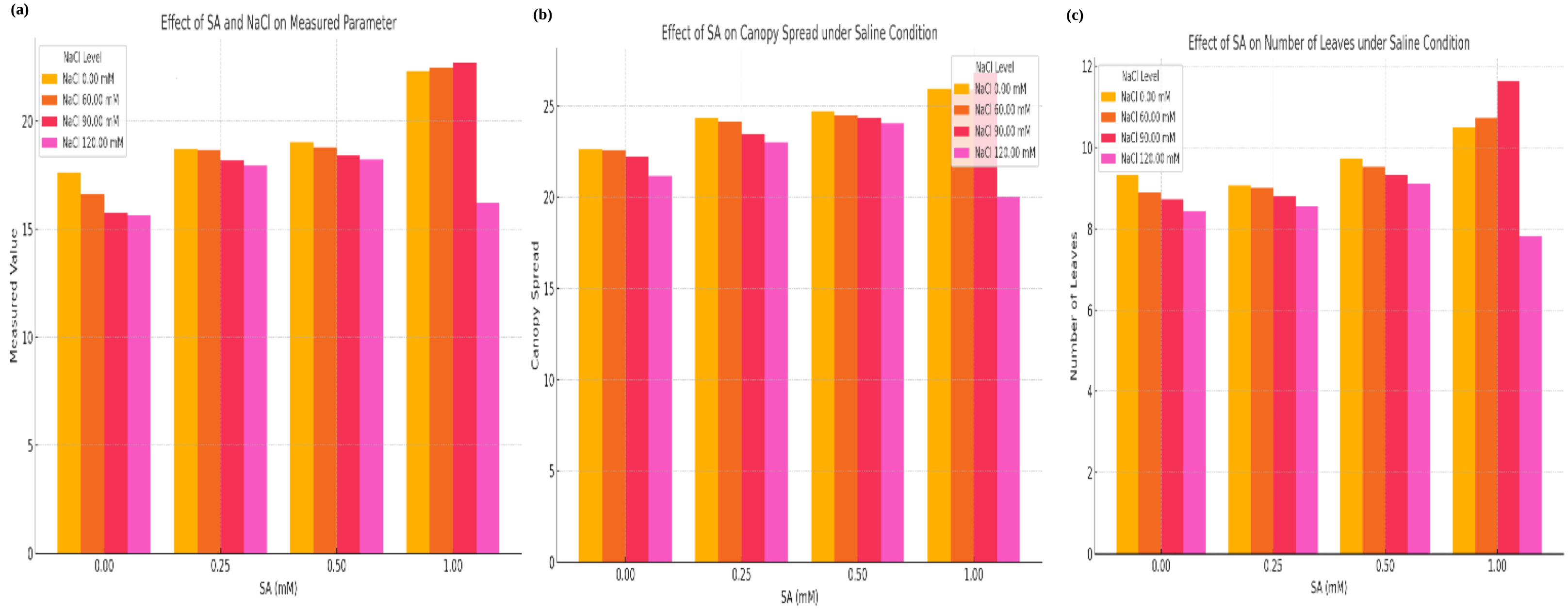

Figure 1. Combined effect of salicylic acid (SA) and salinity (NaCl) stress on morphological traits of plants grown under protected environmental conditions. (a) Effect of SA and NaCl on a measured growth parameter, (b) Effect on canopy spread (cm), (c) Effect on the number of leaves per plant. Each bar represents the mean value of the respective parameter across four salinity levels (0, 60, 90, and 120 mM NaCl) and four SA concentrations (0.00, 0.25, 0.50, and 1.00 mM). The findings of the experiment showed how salicylic acid interacts with other elements to reduce the negative impacts of salt stress on plant growth characteristics. For more clarity, treatments are color-coded. As per the requirement, error bars or alphabetic letters are used to emphasise the significance of variances.

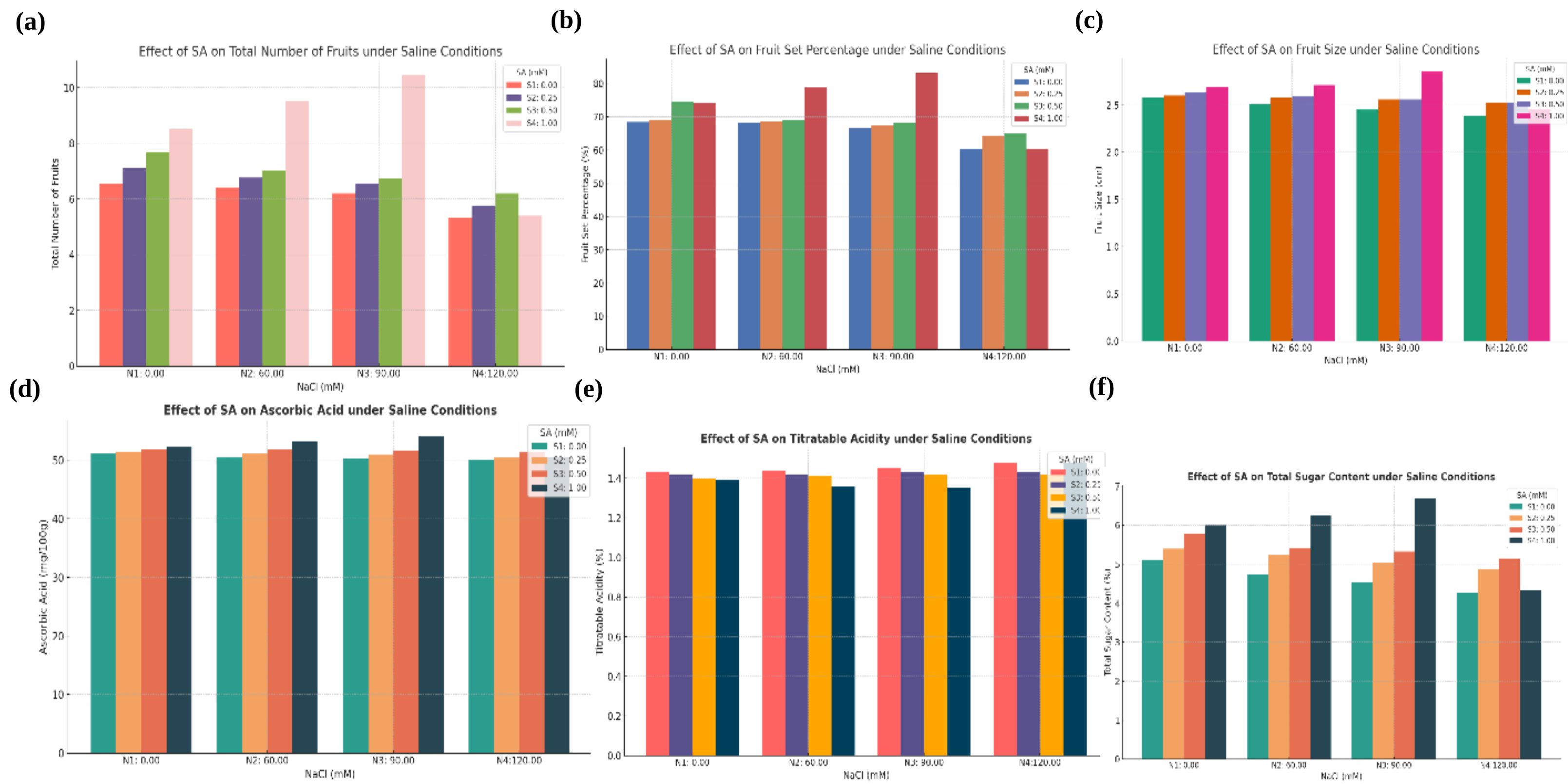

Figure 2. Fruit-related characteristics in saline conditions, such as total No. The values of fruits/plant (a), fruit set % (b), fruit size (c), ascorbic acid content (d), titratable acidity (e), and total sugar content (f) are shown in the figure for different concentrations of NaCl and SA treatments.

Table 2: Effect of salicylic acid (SA) on leaf area, fresh weight, and dry weight of plants under saline conditions in a protected environment.

NaCl (mM)	Leaf Area (S1)	Leaf Area (S2)	Leaf Area (S3)	Leaf Area (S4)	Fresh Weight (S1)	Fresh Weight (S2)	Fresh Weight (S3)	Fresh Weight (S4)	Dry Weight (S1)	Dry Weight (S2)	Dry Weight (S3)	Dry Weight (S4)
0	50.67	52.49	54.98	56.99	23.24	24.25	24.94	27.67	9.96	10.11	10.2	11.64
60	50.18	51.41	53.79	57.07	22.91	23.41	24.35	29.49	9.62	9.78	10.05	11.83
90	49.9	50.47	52.95	58.91	22.45	22.35	24.05	30.31	9.13	9.29	9.82	12.78
120	46.36	49.88	52.52	45.7	20.93	21.91	23.72	21.87	8.53	9.16	9.51	8.09
MEAN	49.28	51.06	53.56	54.67	22.38	22.98	24.27	27.34	9.31	9.58	9.9	11.08

Improves the cellular integrity and metabolic activity during stress. These findings are also in line with previous studies that have indicated salicylic acid -induced stomatal conductance, enzymatic antioxidant activity, and chlorophyll increase (Khodary, 2004; Arfan *et al*., 2007). Additionally, salicylic acid treatment significantly enhanced the reproductive outcomes, such as overall output, blooming and fruit set, which indicated its impact on reproductive stability under salt. The gains are likely to be attributed to the salicylic acid's functions in controlling hormonal balance and enhancing the source-sink relationship in fruit development. Importantly, the use of salicylic acid augmented fruit quality attributes like total sugar contents, ascorbic acid levels, and titratable acidity, which indicated its benefits in augmenting antioxidant capacity and in maintaining metabolic homeostasis (Afrin *et al*., 2016; Aaby *et al*., 2007).

However, when the salinity was so high (120 mM NaCl), and the growth and yield declined regardless of salicylic acid levels, the benefits of salicylic acid were minimized. This means that there is a specific limit of salinity beyond which the salicylic acid protective effect will not be effective due to the ion toxicity and osmotic stress, which will overcome the physiological defense system of the plant (Keutgen and Pawelzik, 2009; Rahimi and Biglarifard, 2011).

The best therapy to increase plant performance under stress is 1.00 mM SA with 90 mM NaCl, as shown by the interaction between salinity and salicylic acid (SA). An increasing concentration of both salicylic acid (SA) and NaCl produced a decreasing or negative effect, whereas a lower concentration of salicylic acid (SA) (0.25 and 0.50 mM) did the same thing to a lesser degree. The outcomes provide hope to the hypothesis that salicylic acid (SA) possesses dose-effective reaction, where optimal concentrations stimulate mechanisms of stress tolerance and supra-optimal concentrations have a serious side effect on hormonal cues or a moderate response to stress (Shakirova *et al*., 2003; Szepesi, 2005).

Finally, at the moderate salinities, the external introduction of salicylic acid, particularly at 1.00 mM, represents a viable agronomic technique to improve strawberry growth, yield and fruit quality. The lower performance of the process at high levels of salinity, however, demonstrates that the combined approach that includes improved irrigation and soil management practices and the use of the chemical priming agents is required. Future research ought to focus on establishing the underlying molecular action of salicylic acid-induced tolerance and assessing its agronomic sustainability in a variety of conditions and different cultivars under high levels of salinity (90 mM NaCl).

4. CONCLUSION

The use of 1.00 mM salicylic acid (SA) improved vital vegetative growth, reproductive traits, and the quality of fruits expressed in terms of ascorbic acid concentration and total sugar levels significantly. Evaluating its long-term agronomic viability in a range of conditions and cultivars. Based on these observations, salicylic acid enhances the tolerance of plants to the environment by altering physiological and biochemical pathways, thereby boosting the growth and yield in salty soils. Nonetheless, the effectiveness of salicylic acid protection diminished with severe salinity stress (120 mM NaCl), which implies that the salicylic acid protection has a limit to its moderating effect. This highlights that to ensure maximum agricultural production, salicylic acid levels and salinity should be manipulated. Finally, 1.00 mM SA is the most appropriate treatment to improve the performance of strawberry plants in the presence of mild salt stress, and it can be proposed as a part of the comprehensive approach towards the control of salt stress. Nevertheless, it is necessary to validate this field-wise to justify its practical use in commercial strawberry cultivation (particularly in salt-impacted areas) and to conduct further studies of the mechanisms of salicylic acid -induced stress tolerance.

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5. Author's Contribution Statement

Fayaz Khan: Identification of research gap, methodology and design of experiment. Qaisar Ali Khan: Data collection, Zia Ul Haq: Helped in finalizing the technical write-up, Irfan Ali: Supervised the research, Tajwar Alam: Reviewed and improved the manuscript, Syed Mudassir Raza: Statistical data analysis. Aksar Ali Khan: Conceptualization and formatting of manuscript, Abu Saad: Reviewing and editing, Muhammad Riaz: Visualisation, Abdul Waqas: Formal data analysis, Ahmed Waqas: Reviewing and editing.

6. Conflict of Interest

The authors declare that they have no conflict of interest.

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